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(54) Title: DIARYL THIOETHERS, COMPOSITIONS AND USES THEREOF

(57) Abstract

The present invention relates to diaryl thioethers, including substituted diphenyl thioethers. Furthermore, the present invention relates to the use of the aforementioned compounds as ligands for cellular receptors, including adenosine and G–protein–coupled receptors. In certain embodiments, the compounds of the present invention are selective ligands, e.g., antagonists or agonists, for a particular family or subtype of adenosine or G–protein–coupled receptor. Additionally, the present invention relates to pharmaceutical preparations comprising the ligands, and their use as medicaments in the treatment of diseases deriving, at least in part, from cellular pathways incorporating adenosine or G–protein–coupled receptors.

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Diaryl Thioethers, Compositions and Uses Thereof

Related Applications

This application claims the benefit of priority to United States Provisional Patent Application serial number 60/125,959, filed March 24, 1999.

Background of the Invention

Eukaryotic organisms are composed of a multitude of cells, tissues and organs that must react rapidly and in a concerted manner to environmental stimuli, including external and internal stimuli, and intercellular and intracellular stimuli. In order for eukaryotic organisms to do so, mechanisms and biochemical pathways for achieving rapid and concerted responses have evolved. Cell surface proteins that span the cell membrane provide a means for achieving these responses.

Cell surface proteins permit intracellular transduction of extracellular signals. Cell surface proteins provide eukaryotic, as well as prokaryotic, cells a means to detect extracellular signals and transduce such signals intracellularly in a manner that ultimately results in a cellular response or a concerted tissue or organ response. Cell surface proteins, by intracellularly transmitting information regarding the extracellular environment via specific intracellular pathways induce an appropriate response to a particular stimulus. The response may be immediate and transient, slow and sustained, or some mixture thereof. By virtue of an array of varied membrane surface proteins, eukaryotic cells are exquisitely sensitive to their environment.

Extracellular signal molecules, such as growth hormones, vasodilators and neurotransmitters, exert their effects, at least in part, via interaction with cell surface proteins. For example, some extracellular signal molecules cause changes in transcription of target gene via changes in the levels of secondary messengers, such as cAMP. Other signals, indirectly alter gene expression by activating the expression of genes, such as immediate-early genes that encode regulatory proteins, which in turn activate expression of other genes that encode transcriptional regulatory proteins. For example, neuron gene expression is modulated by numerous extracellular signals, including neurotransmitters and membrane electrical activity. Transsynaptic signals cause rapid responses in neurons that occur over a period of time ranging from milleseconds, such as the opening of ligandgated channels, to seconds and minutes, such as second messenger-mediated events. Genes in neural cells that are responsive to transsynaptic stimulation and membrane electrical activity, include genes, called immediate early genes, whose transcription is activated rapidly, within minutes, and transiently (see, e.g.,

Sheng et al. (1990) Neuron 4: 477-485), and genes whose expression requires protein synthesis and whose expression is induced or altered over the course of hours.

Cell surface receptors and ion channels are among the cell surface proteins that respond to extracellular signals and initiate the events that lead to this varied gene expression and response. Ion channels and cell surface-localized receptors are ubiquitous and physiologically important cell surface membrane proteins. They play a central role in regulating intracellular levels of various ions and chemicals, many of which are important for cell viability and function.

Cell surface-localized receptors are membrane spanning proteins that bind extracellular signalling molecules or changes in the extracellular environment and transmit the signal via signal transduction pathways to effect a cellular response. Cell surface receptors bind circulating signal polypeptides, such as growth factors and hormones, as the initiating step in the induction of numerous intracellular pathways. Receptors are classified on the basis of the particular type of pathway that is induced. Included among these classes of receptors are those that bind growth factors and have intrinsic tyrosine kinase activity, such as the heparin binding growth factor (HBGF) receptors, and those that couple to effector proteins through guanine nucleotide binding regulatory proteins, which are referred to as G protein coupled receptors and G proteins, respectively.

The G protein transmembrane signaling pathways consist of three proteins: receptors, G proteins and effectors. G proteins, which are the intermediaries in transmembrane signaling pathways, are heterodimers and consist of alpha , beta and gamma subunits. Among the members of a family of G proteins the alpha subunits differ. Functions of G proteins are regulated by the cyclic association of GTP with the alpha subunit followed by hydrolysis of GTP to GDP and dissociation of GDP.

G protein coupled receptors are a diverse class of receptors that mediate signal transduction by binding to G proteins. Signal transduction is initiated via ligand binding to the cell membrane receptor, which stimulates binding of the receptor to the G protein. The receptor G protein interaction releases GDP, which is specifically bound to the G protein, and permits the binding of GTP, which activates the G protein. Activated G protein dissociates from the receptor and activates the effector protein, which regulates the intracellular levels of specific second messengers. Examples of such effector proteins include adenyl cyclase, guanyl cyclase, phospholipase C, and others.

G protein-coupled receptors, which are glycoproteins, are known to share certain structural similarities and homologies (see, e-g., Gilman, A.G., Ann. Rev. Biochem.56: 615-649 (1987), Strader, C.D. et al. The FASEB Journal 3: 1825-1832 (1989), Kobilka, B.K., et al. Nature 329:75-79 (1985) and Young et al. Cell 45: 711-719 (1986)). Among the G protein-coupled receptors that have been identified and cloned are the substance K receptor, the

angiotensin receptor, the alpha - and beta -adrenergic receptors and the serotonin receptors. G protein-coupled receptors share a conserved structural motif. The general and common structural features of the G protein-coupled receptors are the existence of seven hydrophobic stretches of about 20-25 amino acids each surrounded by eight hydrophilic regions of variable length. It has been postulated that each of the seven hydrophobic regions forms a transmembrane alpha helix and the intervening hydrophilic regions form alternately intracellularly and extracellularly exposed loops. The third cytosolic loop between transmembrane domains five and six is the intracellular domain responsible for the interaction with G proteins.

G protein-coupled receptors are known to be inducible. This inducibility was originally described in lower eukaryotes. For example, the cAMP receptor of the cellular slime mold, Dictyostelium, is induced during differentiation (Klein et al., Science 241: 1467-1472 (1988). During the Dictyostelium discoideum differentiation pathway, cAMP, induces high level expression of its G protein-coupled receptor. This receptor transduces the signal to induce the expression of the other genes involved in chemotaxis, which permits multicellular aggregates to align, organize and form stalks (see, Firtel, R.A., et al. Cell 58: 235-239 (1989) and Devreotes, P., Science 245: 1054-1058 (1989)).

Adenosine is a naturally occurring nucleoside which exhibits diverse and potent physiological actions in the cardiovascular, nervous, pulmonary, renal and immune systems. Adenosine has been shown to terminate superventricular tachycardia through blockage of atrioventricular nodal conduction (J. P. DiMarco, et al., (1985) J. Am. Col. Cardiol. 6:417-425, A. Munoz, et al., (1984) Eur. Heart J. 5:735-738). Adenosine is a potent vasodilator except in the kidney and placenta (R. A. Olsson, (1981) Ann. Rev. Physiol. 43:385-395). Adenosine has been implicated as a preventative agent and in treatment of ventricular dysfunction following episodes of regional or global ischemia (M. B. Forman and C. E. Velasco (1991) Cardiovasc. Drugs and Therapy 5:901-908) and in cerebral ischemia (M. C. Evans, et al., (1987) Neurosci. Lett. 83:287, D. K. J. E., Von Lubitz, et al., (1988) Stroke 19:1133).

Specifically, adenosine acts as a signal molecule, is produced by the body, and found in almost every cell. Additional examples of signal molecules include hormones and neurotransmitters. These endogenous molecules are naturally occurring chemicals used by the body to communicate between cells and to coordinate physiological functions. The actions of individual signal molecules are controlled or mediated through specific recognition sites on the surface of cells, called receptors. For adenosine, four receptor subtypes are known (discussed in greater detail below), A1, A2a, A2b, and A3. Each of these is believed responsible for a different function. For example, A1-receptors regulate the rhythm of the

heart, A2a-receptors dilate blood vessels (vasodilation), and there is growing evidence that stimulation of A3-receptors may impart cardioprotection.

Adenosine is known to modulate diverse physiological functions including induction of sedation, vasodilatation, suppression of cardiac rate and contractility, inhibition of platelet aggregability, stimulation of gluconeogenesis and inhibition of lipolysis (see, Stiles, 1986, Trends Pharmacol. Sci. 7: 486-490; Williams, 1987, Ann. Rev. Pharmacol. Toxicol. 27: 315-345; Ramkumar et al., 1988, Prog. Drug. Res. 32: 195-247). Individual subtypes of adenosine receptor inhibit or stimulate adenylate cyclase (Stiles, ibid.; Williams, ibid.). Substantial progress has been made concerning the biochemical and pharmacological properties of these adenosine receptors such as ligand binding characteristics, glycosylation, and regulation. Besides its effects on adenylate cyclase, adenosine has been shown to open potassium channels, reduce flux through calcium channels, and inhibit or stimulate phosphoinositide turnover through receptor-mediated mechanisms (see, Fredholm & Dunwiddie, 1988, Trends Pharmacol. Sci. 9: 130-134; Sebastiao et al., 1990, Br. J. Pharmacol. 100: 55-62; Stiles, 1990, Clin. Res. 38: 10-18; Nakahata et al., 1991, J. Neurochem. 57: 963-969).

The actions of adenosine are mediated through G-protein coupled receptors; the A1, A2a, A2b and A3 adenosine receptors mentioned above. The adenosine receptors were initially classified into A1 and A2 subtypes on the basis of pharmacological criteria and coupling to adenylate cyclase (Van Caulker, D., Muller, M. and Hamprecht, B. (1979) J. Neurochem., 33:999-1003). Further pharmacological classification of adenosine receptors prompted subdivision of the A2 class into A2a and A2b subtypes on the basis of high and low affinity, respectively, for adenosine and the agonists NECA and CGS-21680 (Bruns, R. F., Lu, G. H. and Pugsley, T. A. (1986) Mol. Pharmacol., 29:331-346; Wan, W., Sutherland, G. R. and Geiger, J. D. (1990) J. Neurochem., 55:1763-1771). Molecular cloning and characterization of the human A3 adenosine receptor is described in Salvatore et al., Proc. Natl. Acad. Sci. U.S.A., vol. (90) pp 10365-10369, November 1993. The existence of A1, A2a, A2b and A3 subtypes has been confirmed by cloning and functional characterization of expressed bovine, canine, rat and human receptors. Cloning and characterization of the human A1, A2a, A2b and A3 receptors are described in GB 2264948-A. Based on the use of these cloned receptors, an assay has been described to identify adenosine receptor agonists and antagonists and determine their binding affinity (see GB 2 264 948 A, published Sep. 15, 1993; see also R. F. Bruns, et al., (1983) Proc. Natl. Acad. Sci. U.S.A., 80:2077-2080; R. F. Bruns, et al.,(1986) Mol. Pharmacol., 29:331-346; M. F. Jarvis, et al. (1989) J. Pharma. Exp. Therap., 251:888-893; K. A. Jacobson et al., (1989) J. Med. Chem., 32:1043-1051).

Although adenosine can affect a variety of physiological functions, particular attention has been directed over the years toward actions which might lead to clinical applications. Preeminent has been the cardiovascular effects of adenosine which lead to vasodilation and

hypotension but which also lead to cardiac depression. The antilipolytic, antithrombotic and antispasmodic actions of adenosine have also received some attention. Adenosine stimulates steroidogenesis in adrenal cells, again probably via activation of adenylate cyclase. Adenosine has inhibitory effects on neurotransmission and on spontaneous activity of central neurons. Finally, adenosine is a known bronchoconstrictor.

Methods of treating conditions related to the physiological action of adenosine have, to date, proven inferior due to the lack of selectivity of the compounds among the multiple adenosine receptor subtypes present in whole tissue. (R. F. Bruns et al., (1986) Mol. Pharm., 29:331-346) and the inability to extrapolate activities measured on non-human tissues due to the species variability in the affinity for adenosine analogs and the physiological effects of adenosine (Ukera, et al., (1986) FEBS Lett, 209:122-128, Stone, et al.(1988) 15, 31-46).

Adenosine receptor agonists, antagonists and binding enhancers have been identified and implicated for usage in the treatment of physiological complications resulting from cardiovascular, pulmonary, renal and neurological disorders. The pharmaceutical industry is actively working to develop new drug candidates that bind at one or more adenosine receptor. For review articles, see: Jacobsen and Suzuki, *Drug Development Research* **1996**, *39*, 289-300; and DeNinno, *Annual Reports in Medicinal Chemistry* **1998**, *33*, 111-120. Adenosine receptor agonists have been identified for use as vasodilators ((1989) FASEB. J., 3(4) Abs 4770 and 4773, (19910 J. Med. Chem., (1988) 34:2570), antihypertensive agents (D. G. Taylor et al., FASEB J., (1988) 2:1799), and anti-psychotic agents (T. G. Heffner et al., (1989) Psychopharmacology, 98:31-38). Adenosine receptor agonists have been identified for use in improving renal function (R. D. Murray and P. C. Churchill,(1985) J. Pharmacol. Exp. Therap., 232:189-193). Adenosine receptor allosteric or binding enhancers have shown utility in the treatment of ischemia, seizures or hypoxia of the brain (R. F. Bruns, et al. (1990) Mol. Pharmacol., 38:939-949; C. A. Janusz, et al., (1991) Brain Research, 567:181-187).

The important neurotransmitter serotonin (5-HT) was identified around 50 years ago after it was isolated from whole blood and shown to have vasoconstrictor properties. See, Page, I.H. *Physiol. Rev.* 34, 563 (1954). Its identification in brain led to immediate speculation that it could be involved as a mediator in psychiatric disorders and there is now substantial experimental evidence to demonstrate that this hypothesis was correct. The role of 5-HT in appetite, sleep, mood control, thermoregulation and pain has been extensively reviewed. See, Glennon, R.A. *Neurosci. Biobehav. Rev.* 14, 35 (1990); Wilkinson and Dourish in "Serotonin Receptor Subtypes: Basic and Clinical Aspects," S. Peroutka, Ed.; John Wiley and Sons, New York, 1991, p. 147; Zifa, E. *Pharmacol. Rev.* 44, 401 (1992). The multiple actions of 5-HT are mediated through 14 different receptors and these have been subdivided into 7 distinct groups on the basis of operational pharmacology, sequence analysis

and transduction mechanisms; it is now clear that all 14 human receptors are encoded by different genes. See, Hoyer, D. et al. *Pharmacol. Rev.* 46, 157 (1994); Hartig, P.R. et al. *TiPS* 17, 103 (1996); Gerhardt, C.C. *Eur. J. Pharmacol.* 334, 1 (1997). Many compounds with mixed 5-HT receptor binding profiles have been reported.

Most cerebral functions are the result of the converging actions of many different neurotransmitters. See, Dubovsky et al. *J. Clin. Psychiatry* 56 (suppl. 2), 38 (1995). For example, excitability in the human cortex is regulated by acetylcholine, GABA, norepinephrine, histamine, and purines, in addition to 5-HT. Each of these transmitters may produce more than one postsynaptic signal in the same neuron, and more than one transmitter may induce the same change in postsynaptic neurons. This kind of overlap, like the overlap of 5HT subsystems described below, is not necessarily redundant; it provides a mechanism for fine tuning complex adaptations to multiple kinds of input.

Interactions between serotonergic subsystems and between 5-HT and other neurotransmitters make it possible for 5-HT to contribute to the regulation of many core psychobiological functions that are disrupted in psychiatric disorders, including mood, anxiety, arousal, vigilance, irritability, thinking, cognition, appetites, the sleep-wake cycle, circadian and seasonal rhythms, nociception, and neuroendocrine functions. 5-HT may serve as a "neurochemical brake" on certain innate behaviors, for example, reduced serotonergic tone-releasing behaviors that are normally suppressed, such as aggression. For this reason, evidence of reduced central 5-HT activity, which was originally thought to be specific for depression and then for suicidal depression, turned out to be correlated with violent and/or impulsive unpremeditated suicidal behavior, whether the descriptive diagnosis is depression, schizophrenia, behavior disorder, or personality disorder. Reduced serotonergic tone is not even restricted to suicidality per se; it is associated with loss of control over impulsivity and/or aggression whether it is directed inward or outward. Van Pragg, H.M. *Psychiatry Res.* 34, 1 (1990).

The many functions in which 5-HT participates are mediated by a variety of receptors. See, e.g., Leonard, B.E. *J. Clin. Psychiatry* 54, 3 (1993); Gothert, M. *Ann NY Acad Sci* 604, 102 (1990). 5-HT receptors differ with respect to distribution in the nervous system, structure, location on the presynaptic (cell body or dendritic) or postsynaptic neuron, pharmacology, and second-messenger system. 5-HT heteroreceptors are located on presynaptic neurons utilizing other neurotransmitters, the release of which may be enhanced or reduced by 5-HT. Genes have been identified for the 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, 5-HT_{1F}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_3, 5-HT_{5A}, 5-HT_{5b}, 5-HT_6, and 5-HT_7 receptors. The

multiplicity of 5-HT receptor subtypes makes it possible for 5-HT to participate in the regulation of multiple functions, with different receptors sometimes participating in the regulation of the same function.

The differences among 5-HT receptors impact the design of therapies operating on or through the receptors. For example, the therapeutically relevant effect of a medication may take place on different receptors than those originally postulated. It is not known whether sumatriptan, a 5-HT_{1D} agonist antimigraine drug, acts on the 5-HT_{1d} or the 5-HT_{1D} subtype. Knowing exactly which of these receptors is targeted would facilitate the development of more specific medications that may have fewer adverse effects. Similarly, if an antipsychotic drug believed to act on 5-HT_3 receptors also acts on 5-HT_4 receptors, the question arises of whether a medication that is selective for these would be better for a particular clinical subtype of psychosis. Moreover, substances that act on more than one 5-HT receptor subtype may be more effective than those that act on only one receptor in the treatment of complicated syndromes. Studying medication effects on a growing number of receptors is a painstaking task, but it may eventually lead to the development of medications that are useful in complex and treatment-resistant syndromes.

For example, The 5-HT_2 receptor has been implicated in the pathogenesis and treatment of psychosis, especially schizophrenia. Stimulation of this receptor facilitates dopamine release by amphetamines, which are psychotogenic, and, independently of dopaminergic systems, interferes with sensorimotor gating, reproducing a finding in schizophrenia. Observations that stimulation of the 5-HT_{2C} receptor with the 5-HT agonist *m*-chlorophenylpiperazine (*m*-CPP) worsens psychosis, and that schizophrenic patients with evidence of higher 5-HT receptor responsiveness to *m*-CPP have a better response to the 5-HT_2 antagonist antipsychotic drug clozapine provide further evidence for a role of the 5-HT_2 receptor in some cases of schizophrenia.

"Atypical" antipsychotic drugs are more potent as 5-HT_2 antagonists than as dopamine D_2 receptor antagonists. The 5-HT_2 antagonist antipsychotic drugs share the ability to improve negative as well as positive symptoms of schizophrenia, to be effective in treatment resistant schizophrenia, to produce few extrapyramidal side effects, and to reduce signs of tardive dyskinesia. They appear to be at least as effective as classical neuroleptics for nonrefractory schizophrenia. Kahn, R.S. et al. *Am. J. Psychiatry* 150, 1337 (1993).

The contribution of the 5-HT_3 receptor to mesolimbic dopamine release appears to account for the ability of 5-HT_3 antagonists to inhibit elevated mesolimbic dopamine release and for their effectiveness in states in which abnormal dopaminergic tone has been thought to

play a role, or at least in which reducing dopaminergic transmission may be helpful, such as schizophrenia, mania, substance abuse, and emesis. In addition, 5-HT_3 antagonists enhance acetylcholine release. As a result, ondansetron was found to attenuate cognitive impairment produced by reduced cholinergic transmission in an animal model.

The wide use of new 5-HT receptor-selective agents indicates that their applications are not limited to any specific diagnostic category. However, specific aspects or dimensions of these diagnostic categories may be targeted more precisely. For example, 5-HT_2 antagonist antidepressants may be more effective than other antidepressants against the dimension of severity, which in depression is often linked to anxiety, another dimension linked to this receptor. The 5-HT_2 antagonist antipsychotic drugs seem to have enhanced potency for treating negative symptoms of schizophrenia, which may bear a relationship to depression but do not necessarily reflect the actual presence of comorbid depression. Because negative symptoms may also be improved by adding an SRI to a classical neuroleptic, there is probably more than one serotonergic route to treating the interaction of psychosis with dimensions associated with negative symptoms, such as treatment resistance and possibly neurologic dysfunction. Medications that act on 5-HT_3 receptors may prove most useful for syndromes of psychosis, substance abuse, and/or cognitive dysfunction.

It may also become possible to combine medications acting on different aspects of 5-HT function with greater reliability in the treatment of complex disorders. For example, some patients with chronic psychotic bipolar mood disorders develop irritability, psychosis, and mood cycling while taking antidepressants, only to become more depressed when antidepressants are withdrawn. If the effect of a medication on cycling can be dissected from its effect on mood and psychosis, it might be possible to improve one without making the others worse. This might be accomplished by adding a 5-HT_2 or 5-HT_3 antagonist to a medication selective for a dopamine receptor subtype.

Summary of the Invention

The present invention relates to diaryl thioethers, including substituted diphenyl thioethers. Furthermore, the present invention relates to the use of the aforementioned compounds as ligands for cellular receptors, including adenosine and G-protein-coupled receptors. In certain embodiments, the compounds of the present invention are selective ligands, e.g., antagonists or agonists, for a particular family or subtype of adenosine or G-protein-coupled receptor. Additionally, the present invention relates to pharmaceutical preparations comprising the ligands, and their use as medicaments in the treatment of diseases

deriving, at least in part, from cellular pathways incorporating adenosine or G-protein-coupled receptors.

Brief Description of the Figures

Figure 1 depicts the antibacterial activity of certain compounds of the present invention against methicillin-resistant *Staphylococcus aureus* (MRSA).

Figure 2 depicts the structures and activity profiles against adenosine receptors of three diaryl thioethers that are selective for the human adenosine A_3 receptor.

Figure 3 depicts certain compounds of the present invention and their IC_{50}s against four human adenosine receptors.

Figure 4 depicts certain compounds of the present invention and their IC_{50}s against four human adenosine receptors.

Figure 5 depicts certain compounds of the present invention and their IC_{50}s against a human adenosine receptor and a rat adenosine receptor.

Figure 6 depicts certain compounds of the present invention and their IC_{50}s against a human adenosine receptor and a rat adenosine receptor.

Figure 7 depicts certain compounds of the present invention and their IC_{50}s against a human adenosine receptor and a rat adenosine receptor.

Figure 8 depicts certain compounds of the present invention and their IC_{50}s against a human adenosine receptor and a rat adenosine receptor.

Figure 9 depicts three compounds of the present invention and some of their ADME properties.

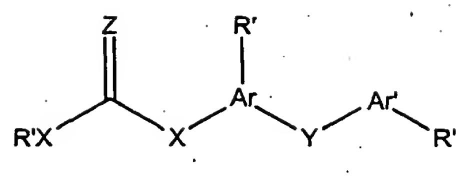
Figure 10 depicts three compounds of the present invention and some of their ADME properties.

Figure 11 depicts three compounds of the present invention and some of their ADME properties.

Detailed Description of the Invention

I. Compounds & Methods of the Invention

In certain embodiments, the compounds of the present invention are represented by general structure **1**:



1

wherein

Ar and Ar' are independently selected from the group consisting of aromatic and heteroaromatic moieties;

X represents independently for each occurrence O, S, Se, NR, PR or AsR;

Y represents O, S, Se, NR, PR or AsR;

Z represents O, S, Se, NR, PR or AsR;

R represents independently for each occurrence hydrogen, optionally substituted alkyl, aryl, or heteroaryl; or formyl, acyl, sulfonyl, -Si(alkyl)$_d$(aryl)$_g$, -Sn(alkyl)$_d$(aryl)$_g$, or -$(CH_2)_mR_8$;

R' represents independently for each occurrence halogen, alkyl, alkenyl, alkynyl, hydroxyl, alkoxyl, silyloxy, amino, nitro, sulfhydryl, alkylthio, imine, amide, phosphoryl, phosphonate, phosphine, carbonyl, carboxyl, carboxamide, anhydride, silyl, thioalkyl, alkylsulfonyl, arylsulfonyl, selenoalkyl, ketone, aldehyde, ester, heteroalkyl, nitrile, guanidine, amidine, acetal, ketal, amine oxide, aryl, heteroaryl, azide, aziridine, carbamate, epoxide, hydroxamic acid, imide, oxime, sulfonamide, thioamide, thiocarbamate, urea, thiourea, or -$(CH_2)_mR_8$;

Ar or Ar' may be unsubstituted beyond X and Y, or Y, respectively, i.e., R' may be absent, or they may be substituted with R' any number of times consistent with the limitations imposed by the rules of valence;

R_8 represents independently for each occurrence an optionally substituted aryl, cycloalkyl, cycloalkenyl, heterocycle or polycycle;

d and g are integers independently selected from the range 0 to 3 inclusive, whose sum is 3; and

m is an integer in the range 0 to 8 inclusive.

In certain embodiments, the compounds of the present invention are represented by **1** and the attendant definitions, wherein X represents NR.

In certain embodiments, the compounds of the present invention are represented by **1** and the attendant definitions, wherein X represents NH.

In certain embodiments, the compounds of the present invention are represented by **1** and the attendant definitions, wherein Y represents S.

In certain embodiments, the compounds of the present invention are represented by **1** and the attendant definitions, wherein Z represents O.

In certain embodiments, the compounds of the present invention are represented by **1** and the attendant definitions, wherein Ar represents phenyl.

In certain embodiments, the compounds of the present invention are represented by **1** and the attendant definitions, wherein there is a single instance of R' on Ar, and said instance of R' represents -C(O)NR_2.

In certain embodiments, the compounds of the present invention are represented by **1** and the attendant definitions, wherein X represents NR; and Y represents S.

In certain embodiments, the compounds of the present invention are represented by **1** and the attendant definitions, wherein X represents NR; and Z represents O.

In certain embodiments, the compounds of the present invention are represented by **1** and the attendant definitions, wherein X represents NR; and Ar represents phenyl.

In certain embodiments, the compounds of the present invention are represented by **1** and the attendant definitions, wherein X represents NR; and there is a single instance of R' on Ar, and said instance of R' represents -C(O)NR_2.

In certain embodiments, the compounds of the present invention are represented by **1** and the attendant definitions, wherein X represents NH; and Y represents S.

In certain embodiments, the compounds of the present invention are represented by **1** and the attendant definitions, wherein X represents NH; and Z represents O.

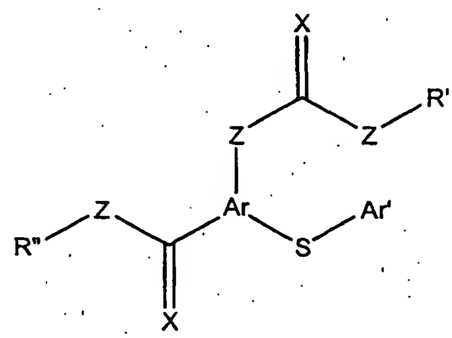
In certain embodiments, the compounds of the present invention are represented by **1** and the attendant definitions, wherein X represents NH; and Ar represents phenyl.

In certain embodiments, the compounds of the present invention are represented by **1** and the attendant definitions, wherein X represents NH; and there is a single instance of R' on Ar, and said instance of R' represents -C(O)NR_2.

In certain embodiments, the compounds of the present invention are represented by **1** and the attendant definitions, wherein Ar represents phenyl; X represents NR; Y represents S; Z represents O; and there is a single instance of R' on Ar, and said instance of R' represents -C(O)NR_2.

In certain embodiments, the compounds of the present invention are represented by **1** and the attendant definitions, wherein Ar represents phenyl; X represents NH; Y represents S; Z represents O; and there is a single instance of R' on Ar, and said instance of R' represents -C(O)NR_2.

In certain embodiments, the compounds of the present invention are represented by general structure **2**:



2

wherein

Ar and Ar' are independently selected from the group consisting of optionally substituted aromatic and heteroaromatic moieties;

X represents independently for each occurrence O, S, Se, NR, PR or AsR;

Z represents independently for each occurrence O, S, Se, NR, PR or AsR;

R represents independently for each occurrence hydrogen, optionally substituted alkyl, aryl, or heteroaryl; or formyl, acyl, sulfonyl, -Si(alkyl)$_d$(aryl)$_g$, -Sn(alkyl)$_d$(aryl)$_g$, or -$(CH_2)_mR_8$;

R' and R" represent independently for each occurrence hydrogen, halogen, alkyl, alkenyl, alkynyl, hydroxyl, alkoxyl, silyloxy, amino, nitro, sulfhydryl, alkylthio, imine, amide, phosphoryl, phosphonate, phosphine, carbonyl, carboxyl, carboxamide, anhydride, silyl, thioalkyl, alkylsulfonyl, arylsulfonyl, selenoalkyl, ketone, aldehyde, ester, heteroalkyl, nitrile, guanidine, amidine, acetal, ketal, amine oxide, aryl, heteroaryl, azide, aziridine, carbamate, epoxide, hydroxamic acid, imide, oxime, sulfonamide, thioamide, thiocarbamate, urea, thiourea, or -$(CH_2)_mR_8$;

R_8 represents independently for each occurrence an optionally substituted aryl, cycloalkyl, cycloalkenyl, heterocycle or polycycle;

d and g are integers independently selected from the range 0 to 3 inclusive, whose sum is 3; and

m is an integer in the range 0 to 8 inclusive.

In certain embodiments, the compounds of the present invention are represented by **2** and the attendant definitions, wherein X represents O.

In certain embodiments, the compounds of the present invention are represented by **2** and the attendant definitions, wherein Z represents NR.

In certain embodiments, the compounds of the present invention are represented by **2** and the attendant definitions, wherein R' represents hydrogen, or optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl.

In certain embodiments, the compounds of the present invention are represented by **2** and the attendant definitions, wherein R" represents hydrogen, hydroxyl, or optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl; or aminoalkyl, amino(heteroalkyl); or ZR" taken together represents hydroxyl or a heterocycle comprising Z and a secondary amine.

In certain embodiments, the compounds of the present invention are represented by **2** and the attendant definitions, wherein Ar represents phenyl.

In certain embodiments, the compounds of the present invention are represented by **2** and the attendant definitions, wherein Ar' represents optionally substituted phenyl, pyridyl, or quinolinyl.

In certain embodiments, the compounds of the present invention are represented by **2** and the attendant definitions, wherein X represents O; and Z represents NR.

In certain embodiments, the compounds of the present invention are represented by **2** and the attendant definitions, wherein X represents O; and R' represents hydrogen, or optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl.

In certain embodiments, the compounds of the present invention are represented by **2** and the attendant definitions, wherein X represents O; and R" represents hydrogen, hydroxyl, or optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl; or aminoalkyl, or amino(heteroalkyl); or ZR" taken together represents hydroxyl or a heterocycle comprising Z and a secondary amine.

In certain embodiments, the compounds of the present invention are represented by **2** and the attendant definitions, wherein X represents O; and Ar represents phenyl.

In certain embodiments, the compounds of the present invention are represented by **2** and the attendant definitions, wherein X represents O; and Ar' represents optionally substituted phenyl, pyridyl, or quinolinyl.

In certain embodiments, the compounds of the present invention are represented by **2** and the attendant definitions, wherein Z represents NR; and R' represents hydrogen, or optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl.

In certain embodiments, the compounds of the present invention are represented by **2** and the attendant definitions, wherein Z represents NR; and R" represents hydrogen, hydroxyl, or optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl; or aminoalkyl, or amino(heteroalkyl); or ZR" taken together represents hydroxyl or a heterocycle comprising Z and a secondary amine.

In certain embodiments, the compounds of the present invention are represented by **2** and the attendant definitions, wherein Z represents NR; and Ar represents phenyl.

In certain embodiments, the compounds of the present invention are represented by **2** and the attendant definitions, wherein Z represents NR; and Ar' represents optionally substituted phenyl, pyridyl, or quinolinyl.

In certain embodiments, the compounds of the present invention are represented by **2** and the attendant definitions, wherein Z represents NH; and R' represents hydrogen, or optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl.

In certain embodiments, the compounds of the present invention are represented by **2** and the attendant definitions, wherein Z represents NH; and R" represents hydrogen, hydroxyl, or optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl; or aminoalkyl, or amino(heteroalkyl); or ZR" taken together represents hydroxyl or a heterocycle comprising Z and a secondary amine.

In certain embodiments, the compounds of the present invention are represented by **2** and the attendant definitions, wherein Z represents NH; and Ar represents phenyl.

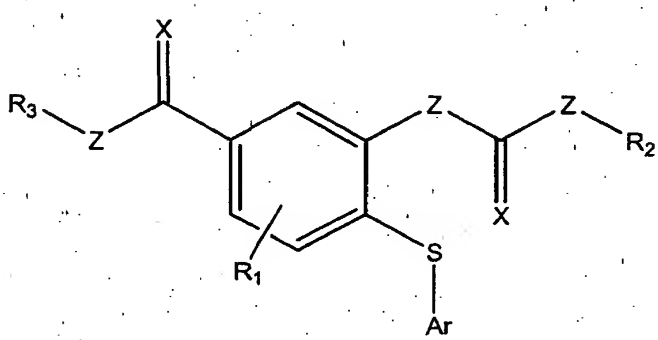
In certain embodiments, the compounds of the present invention are represented by **2** and the attendant definitions, wherein Z represents NH; and Ar' represents optionally substituted phenyl, pyridyl, or quinolinyl.

In certain embodiments, the compounds of the present invention are represented by **2** and the attendant definitions, wherein X represents O; Z represents NR; R' represents hydrogen, or optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl; R" represents hydrogen, hydroxyl, or optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl; or aminoalkyl, or amino(heteroalkyl); or ZR" taken together represents hydroxyl or a heterocycle

comprising Z and a secondary amine; Ar represents phenyl; and Ar' represents optionally substituted phenyl, pyridyl, or quinolinyl.

In certain embodiments, the compounds of the present invention are represented by **2** and the attendant definitions, wherein X represents O; Z represents NH; R' represents hydrogen, or optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl; R" represents hydrogen, hydroxyl, or optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl; or aminoalkyl, or amino(heteroalkyl); or ZR" taken together represents hydroxyl or a heterocycle comprising Z and a secondary amine; Ar represents phenyl; and Ar' represents optionally substituted phenyl, pyridyl, or quinolinyl.

In certain embodiments, the compounds of the present invention are represented by general structure **3**:



3

wherein

Ar is selected from the group consisting of optionally substituted aromatic and heteroaromatic moieties;

X represents independently for each occurrence O, S, Se, NR, PR or AsR;

Z represents independently for each occurrence O, S, Se, NR, PR or AsR;

R represents independently for each occurrence hydrogen, optionally substituted alkyl, aryl, or heteroaryl; or formyl, acyl, sulfonyl, $-Si(alkyl)_d(aryl)_g$, $-Sn(alkyl)_d(aryl)_g$, or $-(CH_2)_mR_8$;

R_1 may be absent, or may be present any number of times consistent with the limitations imposed by the rules of valence;

R_1, when present, represents independently for each occurrence halogen, alkyl, alkenyl, alkynyl, hydroxyl, alkoxyl, silyloxy, amino, nitro, sulfhydryl, alkylthio, imine, amide, phosphoryl, phosphonate, phosphine, carbonyl, carboxyl, carboxamide, anhydride,

silyl, thioalkyl, alkylsulfonyl, arylsulfonyl, selenoalkyl, ketone, aldehyde, ester, heteroalkyl, nitrile, guanidine, amidine, acetal, ketal, amine oxide, aryl, heteroaryl, azide, aziridine, carbamate, epoxide, hydroxamic acid, imide, oxime, sulfonamide, thioamide, thiocarbamate, urea, thiourea, or $-(CH_2)_mR_8$;

R_2 represents hydrogen, optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl; or $-(CH_2)_mR_8$;

R_3 represents hydrogen, hydroxyl, or optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl; or aminoalkyl, or amino(heteroalkyl); or ZR_3 taken together represents hydroxyl or a heterocycle comprising Z and a secondary amine;

R_8 represents independently for each occurrence an optionally substituted aryl, cycloalkyl, cycloalkenyl, heterocycle or polycycle;

d and g are integers independently selected from the range 0 to 3 inclusive, whose sum is 3; and

m is an integer in the range 0 to 8 inclusive.

In certain embodiments, the compounds of the present invention are represented by **3** and the attendant definitions, wherein X represents O.

In certain embodiments, the compounds of the present invention are represented by **3** and the attendant definitions, wherein Z represents NR.

In certain embodiments, the compounds of the present invention are represented by **3** and the attendant definitions, wherein Z represents NH.

In certain embodiments, the compounds of the present invention are represented by **3** and the attendant definitions, wherein R_1 is absent.

In certain embodiments, the compounds of the present invention are represented by **3** and the attendant definitions, wherein R_2 represents optionally substituted aryl or heteroaryl.

In certain embodiments, the compounds of the present invention are represented by **3** and the attendant definitions, wherein R_2 represents 4-trifluorophenyl.

In certain embodiments, the compounds of the present invention are represented by **3** and the attendant definitions, wherein Ar represents optionally substituted phenyl, pyridyl, or quinolinyl.

In certain embodiments, the compounds of the present invention are represented by **3** and the attendant definitions, wherein X represents O; and Z represents NR.

In certain embodiments, the compounds of the present invention are represented by **3** and the attendant definitions, wherein X represents O; and Z represents NH.

In certain embodiments, the compounds of the present invention are represented by **3** and the attendant definitions, wherein X represents O; and R_1 is absent.

In certain embodiments, the compounds of the present invention are represented by **3** and the attendant definitions, wherein X represents O; and R_2 represents optionally substituted aryl or heteroaryl.

In certain embodiments, the compounds of the present invention are represented by **3** and the attendant definitions, wherein X represents O; and R_2 represents 4-trifluorophenyl.

In certain embodiments, the compounds of the present invention are represented by **3** and the attendant definitions, wherein X represents O; and Ar represents optionally substituted phenyl, pyridyl, or quinolinyl.

In certain embodiments, the compounds of the present invention are represented by **3** and the attendant definitions, wherein Z represents NR; and R_1 is absent.

In certain embodiments, the compounds of the present invention are represented by **3** and the attendant definitions, wherein Z represents NR; and R_2 represents optionally substituted aryl or heteroaryl.

In certain embodiments, the compounds of the present invention are represented by **3** and the attendant definitions, wherein Z represents NR; and R_2 represents 4-trifluorophenyl.

In certain embodiments, the compounds of the present invention are represented by **3** and the attendant definitions, wherein Z represents NR; and Ar represents optionally substituted phenyl, pyridyl, or quinolinyl.

In certain embodiments, the compounds of the present invention are represented by **3** and the attendant definitions, wherein Z represents NH; and R_1 is absent.

In certain embodiments, the compounds of the present invention are represented by **3** and the attendant definitions, wherein Z represents NH; and R_2 represents optionally substituted aryl or heteroaryl.

In certain embodiments, the compounds of the present invention are represented by **3** and the attendant definitions, wherein Z represents NH; and R_2 represents 4-trifluorophenyl.

In certain embodiments, the compounds of the present invention are represented by **3** and the attendant definitions, wherein Z represents NH; and Ar represents optionally substituted phenyl, pyridyl, or quinolinyl.

In certain embodiments, the compounds of the present invention are represented by **3** and the attendant definitions, wherein X represents O; Z represents NR; R_1 is absent; R_2

represents optionally substituted aryl or heteroaryl; and Ar represents optionally substituted phenyl, pyridyl, or quinolinyl.

In certain embodiments, the compounds of the present invention are represented by **3** and the attendant definitions, wherein X represents O; Z represents NH; R_1 is absent; R_2 represents 4-trifluorophenyl; and Ar represents optionally substituted phenyl, pyridyl, or quinolinyl.

In certain embodiments, the compounds of the present invention are represented by **3** and the attendant definitions, wherein X represents O; Z represents NR; R_1 is absent; R_2 represents 4-trifluorophenyl; and Ar represents optionally substituted phenyl, pyridyl, or quinolinyl.

In certain embodiments, the compounds of the present invention are represented by **3** and the attendant definitions, wherein X represents O; Z represents NH; R_1 is absent; R_2 represents optionally substituted aryl or heteroaryl; and Ar represents optionally substituted phenyl, pyridyl, or quinolinyl.

In certain embodiments, the present invention relates to ligands for G-protein-coupled receptors, wherein the ligands are represented by any of generalized structures **1**, **2**, or **3**, and any of the sets of definitions associated with one of those structures. Preferably the ligands of the present invention are antagonists or agonists of the G-protein-coupled receptors. In any event, the ligands of the present invention preferably exert their effect on the receptors at a concentration less than about 10 micromolar, more preferably at a concentration less than about 1 micromolar, and most preferably at a concentration less than 100 nanomolar. In certain embodiments, the ligands of the present invention bind selectively to a single family of G-protein-coupled receptors, e.g., the family of adenosine, dopamine or 5-HT receptors. In other embodiments, the ligands of the present invention bind selectively to a subtype of receptor within a family of G-protein-coupled receptors, e.g., the adenosine A_{2a} or 5-HT_{1B} receptor subtype.

In certain embodiments, the selectivity of a ligand for a specific family or subtype of receptor renders that ligand an effective therapeutic agent for an acute or chronic ailment, disease or malady. In certain embodiments, the selectivity of a ligand for a specific family or subtype of receptor consists of a binding affinity for that family or subtype of receptor at least a factor of ten greater than its binding affinity for other families or subtypes of G-protein-coupled receptors. In preferred embodiments, the selectivity of a ligand for a specific family or subtype of receptor consists of a binding affinity for that family or subtype of receptor at least a factor of one hundred greater than its binding affinity for other families or subtypes of G-protein-coupled receptors. In certain embodiments, the selectivity of a ligand for a specific family or subtype of receptor consists of a binding affinity for that family or subtype of

receptor at least a factor of one thousand greater than its binding affinity for other families or subtypes of G-protein-coupled receptors.

The present invention contemplates pharmaceutical formulations (see below) of the ligands of the present invention. In certain embodiments, the pharmaceutical formulations will comprise ligands of the present invention that effect only a specific family or subtype of G-protein-coulped receptor, and thereby have a therapeutic effect on an acute or chronic ailment, disease or malady that is at least in part due to biochemical or physiological processes associated with the receptor(s). In preferred embodiments, the pharmaceutical formulations will comprise ligands of the present invention that effect only a subtype of receptor, e.g., an adenosine or dopamine receptor, and thereby have a therapeutic effect on an acute or chronic ailment, disease or malady that is at least in part due to biochemical or physiological processes associated with the specific subtype of receptor. The *Background of the Invention* (see above) teaches examples of acute or chronic ailments, diseases or maladies that are caused or exacerbated by biochemical or physiological processes associated with specific G-protein-coupled receptors. One of ordinary skill in the art will be able to accumulate, by reference to the scientific literature, a more comprehensive list of acute or chronic ailments, diseases or maladies that are caused or exacerbated by biochemical or physiological processes associated with specific G-protein-coupled receptors. The present invention contemplates pharmaceutical formulations of ligands of the present invention that will be of medicinal value against the aforementioned acute or chronic ailments, diseases or maladies.

II. Definitions

For convenience, certain terms employed in the specification, examples, and appended claims are collected here.

The term "recombinant cells" includes any cells that have been modified by the introduction of heterologous DNA.

The term "control cells" includes cells that are substantially identical to the recombinant cells, but do not express the one or more of the proteins encoded by the heterologous DNA.

The term "heterologous DNA" includes DNA that does not occur naturally as part of the genome in which it is present or which is found in a location or locations in the genome that differs from that in which it occurs in nature. Heterologous DNA is not endogenous to the cell into which it is introduced, but has been obtained from another cell. Heterologous DNA may also be referred to as foreign DNA. Any DNA that one of skill in the art would

recognize or consider as heterologous or foreign to the cell in which is expressed is herein encompassed by heterologous DNA. Examples of heterologous DNA include, but are not limited to, DNA that encodes receptors, reporter genes, transcriptional and transnational regulatory sequences, selectable or traceable marker proteins, such as a protein that confers drug resistance.

The term "cell surface proteins" includes molecules that occur on the surface of cells, interact with the extracellular environment, and transmit or transduce information regarding the environment intracellularly.

The term "extracellular signals" includes a molecule or a change in the environment that is transduced intracellularly via cell surface proteins that interact, directly or indirectly, with the signal. An extracellular signal is any compound or substance that in some manner specifically alters the activity of a cell surface protein. Examples of such signals include, but are not limited to, molecules such as acetylcholine, growth factors, hormones and other mitogenic substances, such as phorbol mistric acetate (PMA), that bind to cell surface receptors and ion channels and modulate the activity of such receptors and channels. Extracellular signals also includes as yet unidentified substances that modulate the activity of a cell surface protein and thereby affect intracellular functions and that are potential pharmacological agents that may be used to treat specific diseases by modulating the activity of specific cell surface receptors.

The term "reporter gene construct" refers to a DNA molecule that includes a reporter gene operatively linked to a transcriptional control sequence. Transcription of the reporter gene is controlled by these sequences. The activity of at least one or more of these control sequences is directly or indirectly regulated by the cell surface protein. The transcriptional control sequence includes the promoter and other regulatory regions, such as enhancer sequences, that modulate the activity of the promoter, or control sequences that modulate the activity or efficiency of the RNA polymerase that recognizes the promoter, or control sequences are recognized by effector molecules, including those that are specifically induced by interaction of an extracellular signal with a cell surface protein. For example, modulation of the activity of the promoter may be effected by altering the RNA polymerase binding to the promoter region, or, alternatively, by interfering with initiation of transcription or elongation of the mRNA. Such sequences are herein collectively referred to as transcriptional control elements or sequences. In addition, the construct may include sequences of nucleotides that alter translation of the resulting mRNA, thereby altering the amount of reporter gene product.

The term "promoter" refers to the region of DNA that is upstream with respect to the direction of transcription of the transcription initiation site. It includes the RNA polymerase binding and transcription imitation sites and any other regions, including, but not limited to repressor or activator protein binding sites, calcium or cAMP responsive sites, and any such

sequences of nucleotides known to those of skill in the art to alter the amount of transcription from the promoter, either directly or indirectly.

The term "promoter that is regulated or mediated by the activity of a cell surface protein" refers to a promoter whose activity changes when a cell is exposed to a particular extracellular signal by virtue of the presence of cell surface proteins whose activities are affected by the extracellular protein. For example,. the c-fos promoter, which is specifically activated upon the specific interaction of certain extracellular signals, such as growth hormones, with a cell surface protein, such as a growth hormone receptor. In particular, the regulation of such promoters by the cell surface protein, though indirect, occurs within minutes of the interaction of the cell surface protein with the extracellular signal. A s used herein, operative linkage refers to a DNA fragment, such as linkage of a promoter to a DNA molecule that is transcribed by RNA polymerase that binds to the promoter, such that the regulatory region is properly positioned for its activity. Thus, a DNA fragment in operative linkage with a promoter is downstream, with respect to the direction of transcription, from the promoter, is in the correct reading frame with respect to the transcription initiation site and is inserted in a manner such transcription elongation proceeds through the DNA fragment.

The term "prodrug" is intended to encompass compounds which, under physiological conditions, are converted into the antibacterial agents of the present invention. A common method for making a prodrug is to select moieties, e.g., for any of the R_1-R_5 substituents of formula **1**, which are hydrolyzed under physiological conditions to provide the desired.' In other embodiments, the prodrug is converted by an enzymatic activity of the host animal or the target bacteria.

The term "heteroatom" as used herein means an atom of any element other than carbon or hydrogen. Preferred heteroatoms are boron, nitrogen, oxygen, phosphorus, sulfur and selenium.

The term "electron-withdrawing group" is recognized in the art, and denotes the tendency of a substituent to attract valence electrons from neighboring atoms, i.e., the substituent is electronegative with respect to neighboring atoms. A quantification of the level of electron-withdrawing capability is given by the Hammett sigma (σ) constant. This well known constant is described in many references, for instance, J. March, <u>Advanced Organic Chemistry</u>, McGraw Hill Book Company, New York, (1977 edition) pp. 251-259. The Hammett constant values are generally negative for electron donating groups ($\sigma[P] = -0.66$ for NH_2) and positive for electron withdrawing groups ($\sigma[P] = 0.78$ for a nitro group), $\sigma[P]$ indicating para substitution. Exemplary electron-withdrawing groups include nitro, acyl, formyl, sulfonyl, trifluoromethyl, cyano, chloride, and the like. Exemplary electron-donating groups include amino, methoxy, and the like.

The term "alkyl" refers to the radical of saturated aliphatic groups, including straight-chain alkyl groups, branched-chain alkyl groups, cycloalkyl (alicyclic) groups, alkyl substituted cycloalkyl groups, and cycloalkyl substituted alkyl groups. In preferred embodiments, a straight chain or branched chain alkyl has 30 or fewer carbon atoms in its backbone (e.g., C_1-C_{30} for straight chain, C_3-C_{30} for branched chain), and more preferably 20 or fewer. Likewise, preferred cycloalkyls have from 3-10 carbon atoms in their ring structure, and more preferably have 5, 6 or 7 carbons in the ring structure.

Moreover, the term "alkyl" (or "lower alkyl") as used throughout the specification, examples, and claims is intended to include both "unsubstituted alkyls" and "substituted alkyls", the latter of which refers to alkyl moieties having substituents replacing a hydrogen on one or more carbons of the hydrocarbon backbone. Such substituents can include, for example, a halogen, a hydroxyl, a carbonyl (such as a carboxyl, an alkoxycarbonyl, a formyl, or an acyl), a thiocarbonyl (such as a thioester, a thioacetate, or a thioformate), an alkoxyl, a phosphoryl, a phosphonate, a phosphinate, an amino, an amido, an amidine, an imine, a cyano, a nitro, an azido, a sulfhydryl, an alkylthio, a sulfate, a sulfonate, a sulfamoyl, a sulfonamido, a sulfonyl, a heterocyclyl, an aralkyl, or an aromatic or heteroaromatic moiety. It will be understood by those skilled in the art that the moieties substituted on the hydrocarbon chain can themselves be substituted, if appropriate. For instance, the substituents of a substituted alkyl may include substituted and unsubstituted forms of amino, azido, imino, amido, phosphoryl (including phosphonate and phosphinate), sulfonyl (including sulfate, sulfonamido, sulfamoyl and sulfonate), and silyl groups, as well as ethers, alkylthios, carbonyls (including ketones, aldehydes, carboxylates, and esters), $-CF_3$, -CN and the like. Exemplary substituted alkyls are described below. Cycloalkyls can be further substituted with alkyls, alkenyls, alkoxys, alkylthios, aminoalkyls, carbonyl-substituted alkyls, $-CF_3$, -CN, and the like.

The term "aralkyl", as used herein, refers to an alkyl group substituted with an aryl group (e.g., an aromatic or heteroaromatic group).

The terms "alkenyl" and "alkynyl" refer to unsaturated aliphatic groups analogous in length and possible substitution to the alkyls described above, but that contain at least one double or triple bond respectively.

Unless the number of carbons is otherwise specified, "lower alkyl" as used herein means an alkyl group, as defined above, but having from one to ten carbons, more preferably from one to six carbon atoms in its backbone structure. Likewise, "lower alkenyl" and "lower alkynyl" have similar chain lengths. Preferred alkyl groups are lower alkyls. In preferred embodiments, a substituent designated herein as alkyl is a lower alkyl.

The term "aryl" as used herein includes 5-, 6- and 7-membered single-ring aromatic groups that may include from zero to four heteroatoms, for example, benzene,

pyrrole, furan, thiophene, imidazole, oxazole, thiazole, triazole, pyrazole, pyridine, pyrazine, pyridazine and pyrimidine, and the like. Those aryl groups having heteroatoms in the ring structure may also be referred to as "aryl heterocycles" or "heteroaromatics." The aromatic ring can be substituted at one or more ring positions with such substituents as described above, for example, halogen, azide, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, alkoxyl, amino, nitro, sulfhydryl, imino, amido, phosphonate, phosphinate, carbonyl, carboxyl, silyl, ether, alkylthio, sulfonyl, sulfonamido, ketone, aldehyde, ester, heterocyclyl, aromatic or heteroaromatic moieties, $-CF_3$, -CN, or the like. The term "aryl" also includes polycyclic ring systems having two or more cyclic rings in which two or more carbons are common to two adjoining rings (the rings are "fused rings") wherein at least one of the rings is aromatic, e.g., the other cyclic rings can be cycloalkyls, cycloalkenyls, cycloalkynyls, aryls and/or heterocyclyls.

The terms *ortho*, *meta* and *para* apply to 1,2-, 1,3- and 1,4-disubstituted benzenes, respectively. For example, the names 1,2-dimethylbenzene and *ortho*-dimethylbenzene are synonymous.

The terms "heterocyclyl" or "heterocyclic group" refer to 3- to 10-membered ring structures, more preferably 3- to 7-membered rings, whose ring structures include one to four heteroatoms. Heterocycles can also be polycycles. Heterocyclyl groups include, for example, thiophene, thianthrene, furan, pyran, isobenzofuran, chromene, xanthene, phenoxathiin, pyrrole, imidazole, pyrazole, isothiazole, isoxazole, pyridine, pyrazine, pyrimidine, pyridazine, indolizine, isoindole, indole, indazole, purine, quinolizine, isoquinoline, quinoline, phthalazine, naphthyridine, quinoxaline, quinazoline, cinnoline, pteridine, carbazole, carboline, phenanthridine, acridine, pyrimidine, phenanthroline, phenazine, phenarsazine, phenothiazine, furazan, phenoxazine, pyrrolidine, oxolane, thiolane, oxazole, piperidine, piperazine, morpholine, lactones, lactams such as azetidinones and pyrrolidinones, sultams, sultones, and the like. The heterocyclic ring can be substituted at one or more positions with such substituents as described above, as for example, halogen, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, amino, nitro, sulfhydryl, imino, amido, phosphonate, phosphinate, carbonyl, carboxyl, silyl, ether, alkylthio, sulfonyl, ketone, aldehyde, ester, a heterocyclyl, an aromatic or heteroaromatic moiety, $-CF_3$, -CN, or the like.

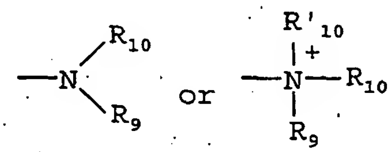
The terms "polycyclyl" or "polycyclic group" refer to two or more rings (e.g., cycloalkyls, cycloalkenyls, cycloalkynyls, aryls and/or heterocyclyls) in which two or more carbons are common to two adjoining rings, e.g., the rings are "fused rings". Rings that are joined through non-adjacent atoms are termed "bridged" rings. Each of the rings of the polycycle can be substituted with such substituents as described above, as for example, halogen, alkyl, aralkyl, alkenyl, alkynyl, cycloalkyl, hydroxyl, amino, nitro, sulfhydryl, imino,

amido, phosphonate, phosphinate, carbonyl, carboxyl, silyl, ether, alkylthio, sulfonyl, ketone, aldehyde, ester, a heterocyclyl, an aromatic or heteroaromatic moiety, $-CF_3$, -CN, or the like.

The term "carbocycle", as used herein, refers to an aromatic or non-aromatic ring in which each atom of the ring is carbon.

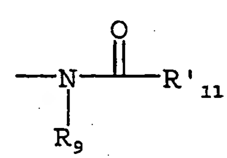
As used herein, the term "nitro" means $-NO_2$; the term "halogen" designates -F, -Cl, -Br or -I; the term "sulfhydryl" means -SH; the term "hydroxyl" means -OH; and the term "sulfonyl" means $-SO_2-$.

The terms "amine" and "amino" are art-recognized and refer to both unsubstituted and substituted amines, e.g., a moiety that can be represented by the general formula:



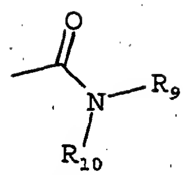
wherein R_9, R_{10} and R'_{10} each independently represent a hydrogen, an alkyl, an alkenyl, $-(CH_2)_m-R_8$, or R_9 and R_{10} taken together with the N atom to which they are attached complete a heterocycle having from 4 to 8 atoms in the ring structure; R_8 represents an aryl, a cycloalkyl, a cycloalkenyl, a heterocycle or a polycycle; and m is zero or an integer in the range of 1 to 8. In preferred embodiments, only one of R_9 or R_{10} can be a carbonyl, e.g., R_9, R_{10} and the nitrogen together do not form an imide. In even more preferred embodiments, R_9 and R_{10} (and optionally R'_{10}) each independently represent a hydrogen, an alkyl, an alkenyl, or $-(CH_2)_m-R_8$. Thus, the term "alkylamine" as used herein means an amine group, as defined above, having a substituted or unsubstituted alkyl attached thereto, i.e., at least one of R_9 and R_{10} is an alkyl group.

The term "acylamino" is art-recognized and refers to a moiety that can be represented by the general formula:



wherein R_9 is as defined above, and R'_{11} represents a hydrogen, an alkyl, an alkenyl or $-(CH_2)_m-R_8$, where m and R_8 are as defined above.

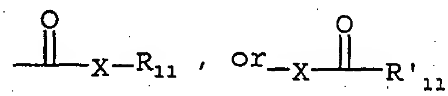
The term "amido" is art recognized as an amino-substituted carbonyl and includes a moiety that can be represented by the general formula:



wherein R_9, R_{10} are as defined above. Preferred embodiments of the amide will not include imides which may be unstable.

The term "alkylthio" refers to an alkyl group, as defined above, having a sulfur radical attached thereto. In preferred embodiments, the "alkylthio" moiety is represented by one of -S-alkyl, -S-alkenyl, -S-alkynyl, and -S-$(CH_2)_m$-R_8, wherein m and R_8 are defined above. Representative alkylthio groups include methylthio, ethyl thio, and the like.

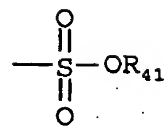
The term "carbonyl" is art recognized and includes such moieties as can be represented by the general formula:



wherein X is a bond or represents an oxygen or a sulfur, and R_{11} represents a hydrogen, an alkyl, an alkenyl, -$(CH_2)_m$-R_8 or a pharmaceutically acceptable salt, R'_{11} represents a hydrogen, an alkyl, an alkenyl or -$(CH_2)_m$-R_8, where m and R_8 are as defined above. Where X is an oxygen and R_{11} or R'_{11} is not hydrogen, the formula represents an "ester". Where X is an oxygen, and R_{11} is as defined above, the moiety is referred to herein as a carboxyl group, and particularly when R_{11} is a hydrogen, the formula represents a "carboxylic acid". Where X is an oxygen, and R'_{11} is hydrogen, the formula represents a "formate". In general, where the oxygen atom of the above formula is replaced by sulfur, the formula represents a "thiolcarbonyl" group. Where X is a sulfur and R_{11} or R'_{11} is not hydrogen, the formula represents a "thiolester." Where X is a sulfur and R_{11} is hydrogen, the formula represents a "thiolcarboxylic acid." Where X is a sulfur and R_{11}' is hydrogen, the formula represents a "thiolformate." On the other hand, where X is a bond, and R_{11} is not hydrogen, the above formula represents a "ketone" group. Where X is a bond, and R_{11} is hydrogen, the above formula represents an "aldehyde" group.

The terms "alkoxyl" or "alkoxy" as used herein refers to an alkyl group, as defined above, having an oxygen radical attached thereto. Representative alkoxyl groups include methoxy, ethoxy, propyloxy, tert-butoxy and the like. An "ether" is two hydrocarbons covalently linked by an oxygen. Accordingly, the substituent of an alkyl that renders that alkyl an ether is or resembles an alkoxyl, such as can be represented by one of -O-alkyl, -O-alkenyl, -O-alkynyl, -O-$(CH_2)_m$-R_8, where m and R_8 are described above.

The term "sulfonate" is art recognized and includes a moiety that can be represented by the general formula:

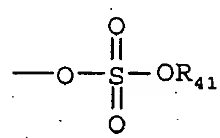


in which R_{41} is an electron pair, hydrogen, alkyl, cycloalkyl, or aryl.

The terms triflyl, tosyl, mesyl, and nonaflyl are art-recognized and refer to trifluoromethanesulfonyl, *p*-toluenesulfonyl, methanesulfonyl, and nonafluorobutanesulfonyl groups, respectively. The terms triflate, tosylate, mesylate, and nonaflate are art-recognized and refer to trifluoromethanesulfonate ester, *p*-toluenesulfonate ester, methanesulfonate ester, and nonafluorobutanesulfonate ester functional groups and molecules that contain said groups, respectively.

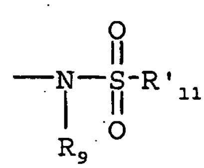
The abbreviations Me, Et, Ph, Tf, Nf, Ts, Ms represent methyl, ethyl, phenyl, trifluoromethanesulfonyl, nonafluorobutanesulfonyl, *p*-toluenesulfonyl and methanesulfonyl, respectively. A more comprehensive list of the abbreviations utilized by organic chemists of ordinary skill in the art appears in the first issue of each volume of the *Journal of Organic Chemistry*; this list is typically presented in a table entitled Standard List of Abbreviations. The abbreviations contained in said list, and all abbreviations utilized by organic chemists of ordinary skill in the art are hereby incorporated by reference.

The term "sulfate" is art recognized and includes a moiety that can be represented by the general formula:



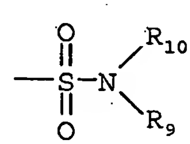
in which R_{41} is as defined above.

The term "sulfonamido" is art recognized and includes a moiety that can be represented by the general formula:



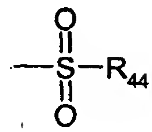
in which R_9 and R'_{11} are as defined above.

The term "sulfamoyl" is art-recognized and includes a moiety that can be represented by the general formula:



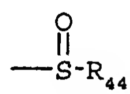
in which R_9 and R_{10} are as defined above.

The term "sulfonyl", as used herein, refers to a moiety that can be represented by the general formula:



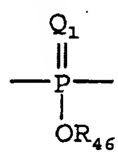
in which R_{44} is selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclyl, aryl, or heteroaryl.

The term "sulfoxido" as used herein, refers to a moiety that can be represented by the general formula:

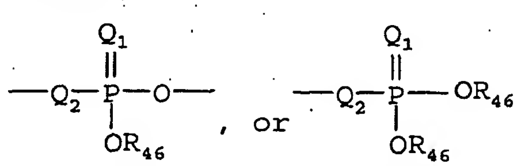


in which R_{44} is selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclyl, aralkyl, or aryl.

A "phosphoryl" can in general be represented by the formula:

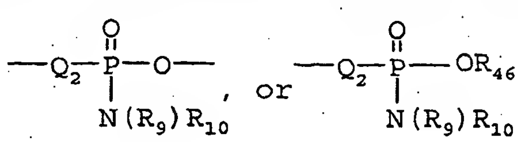


wherein Q_1 represented S or O, and R_{46} represents hydrogen, a lower alkyl or an aryl. When used to substitute, e.g., an alkyl, the phosphoryl group of the phosphorylalkyl can be represented by the general formula:



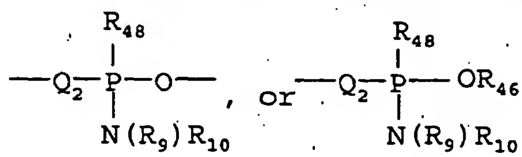
wherein Q_1 represented S or O, and each R_{46} independently represents hydrogen, a lower alkyl or an aryl, Q_2 represents O, S or N. When Q_1 is an S, the phosphoryl moiety is a "phosphorothioate".

A "phosphoramidite" can be represented in the general formula:



wherein R_9 and R_{10} are as defined above, and Q_2 represents O, S or N.

A "phosphonamidite" can be represented in the general formula:



wherein R_9 and R_{10} are as defined above, Q_2 represents O, S or N, and R_{48} represents a lower alkyl or an aryl, Q_2 represents O, S or N.

A "selenoalkyl" refers to an alkyl group having a substituted seleno group attached thereto. Exemplary "selenoethers" which may be substituted on the alkyl are selected from one of -Se-alkyl, -Se-alkenyl, -Se-alkynyl, and -Se-$(CH_2)_m$-R_7, m and R_7 being defined above.

Analogous substitutions can be made to alkenyl and alkynyl groups to produce, for example, aminoalkenyls, aminoalkynyls, amidoalkenyls, amidoalkynyls, iminoalkenyls, iminoalkynyls, thioalkenyls, thioalkynyls, carbonyl-substituted alkenyls or alkynyls.

As used herein, the definition of each expression, e.g. alkyl, m, n, etc., when it occurs more than once in any structure, is intended to be independent of its definition elsewhere in the same structure.

It will be understood that "substitution" or "substituted with" includes the implicit proviso that such substitution is in accordance with permitted valence of the substituted atom and the substituent, and that the substitution results in a stable compound, e.g., which does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, etc.

As used herein, the term "substituted" is contemplated to include all permissible substituents of organic compounds. In a broad aspect, the permissible substituents include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, aromatic and nonaromatic substituents of organic compounds. Illustrative substituents include, for example, those described herein above. The permissible substituents can be one or more and the same or different for appropriate organic compounds. For purposes of this invention, the heteroatoms such as nitrogen may have hydrogen substituents and/or any permissible substituents of organic compounds described herein which satisfy the valences of the heteroatoms. This invention is not intended to be limited in any manner by the permissible substituents of organic compounds.

The phrase "protecting group" as used herein means temporary substituents which protect a potentially reactive functional group from undesired chemical transformations. Examples of such protecting groups include esters of carboxylic acids, silyl ethers of alcohols, and acetals and ketals of aldehydes and ketones, respectively. The field of protecting group

chemistry has been reviewed (Greene, T.W.; Wuts, P.G.M. *Protective Groups in Organic Synthesis*, 2nd ed.; Wiley: New York, 1991).

Certain compounds of the present invention may exist in particular geometric or stereoisomeric forms. The present invention contemplates all such compounds, including *cis-* and *trans-*isomers, *R-* and *S-*enantiomers, diastereomers, (D)-isomers, (L)-isomers, the racemic mixtures thereof, and other mixtures thereof, as falling within the scope of the invention. Additional asymmetric carbon atoms may be present in a substituent such as an alkyl group. All such isomers, as well as mixtures thereof, are intended to be included in this invention.

If, for instance, a particular enantiomer of a compound of the present invention is desired, it may be prepared by asymmetric synthesis, or by derivation with a chiral auxiliary, where the resulting diastereomeric mixture is separated and the auxiliary group cleaved to provide the pure desired enantiomers. Alternatively, where the molecule contains a basic functional group, such as amino, or an acidic functional group, such as carboxyl, diastereomeric salts are formed with an appropriate optically-active acid or base, followed by resolution of the diastereomers thus formed by fractional crystallization or chromatographic means well known in the art, and subsequent recovery of the pure enantiomers.

Contemplated equivalents of the compounds described above include compounds which otherwise correspond thereto, and which have the same general properties thereof (e.g. the ability to bind to opioid receptors), wherein one or more simple variations of substituents are made which do not adversely affect the efficacy of the compound in binding to receptors. In general, the compounds of the present invention may be prepared by the methods illustrated in the general reaction schemes as, for example, described below, or by modifications thereof, using readily available starting materials, reagents and conventional synthesis procedures. In these reactions, it is also possible to make use of variants which are in themselves known, but are not mentioned here.

For purposes of this invention, the chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, Handbook of Chemistry and Physics, 67th Ed., 1986-87, inside cover. Also for purposes of this invention, the term "hydrocarbon" is contemplated to include all permissible compounds having at least one hydrogen and one carbon atom. In a broad aspect, the permissible hydrocarbons include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, aromatic and nonaromatic organic compounds which can be substituted or unsubstituted.

The term "ED_{50}" means the dose of a drug which produces 50% of its maximum response or effect. Alternatively, the dose which produces a pre-determined response in 50% of test subjects or preparations.

The term "LD_{50}" means the dose of a drug which is lethal in 50% of test subjects.

The term "therapeutic index" refers to the therapeutic index of a drug defined as LD_{50}/ED_{50}.

The term "structure-activity relationship (SAR)" refers to the way in which altering the molecular structure of drugs alters their interaction with a receptor, enzyme, etc.

The term "agonist" refers to a compound that mimics the action of natural transmitter or, when the natural transmitter is not known, causes changes at the receptor complex in the absence of other receptor ligands.

The term "antagonist" refers to a compound that binds to a receptor site, but does not cause any physiological changes unless another receptor ligand is present.

The term "competitive antagonist" refers to a compound that binds to a receptor site; its effects can be overcome by increased concentration of the agonist.

The term "partial agonist" refers to a compound that binds to a receptor site but does not produce the maximal effect regardless of its concentration.

The term "ligand" refers to a compound that binds at the receptor site.

III. Biochemical Activity at G-Protein-Coupled Receptors, and Assays to Detect That Activity

Assaying processes are well known in the art in which a reagent is added to a sample, and measurements of the sample and reagent are made to identify sample attributes stimulated by the reagent. For example, one such assay process concerns determining in a chromogenic assay the amount of an enzyme present in a biological sample or solution. Such assays are based on the development of a colored product in the reaction solution. The reaction develops as the enzyme catalyzes the conversion of a colorless chromogenic substrate to a colored product.

Assays useful in the present invention concern determining the activity of receptors the activation of which initiates subsequent intracellular events in which intracellular stores of calcium ions are released for use as a second messenger. Activation of some G-protein-coupled receptors stimulates the formation of inositol triphosphate (IP3, a G-protein-coupled receptor second messenger) through phospholipase C-mediated hydrolysis of phosphatidylinositol, Berridge and Irvine (1984). Nature 312:315-21. IP3 in turn stimulates the release of intracellular calcium ion stores.

A change in cytoplasmic calcium ion levels caused by release of calcium ions from intracellular stores is used to determine G-protein-coupled receptor function. This is another type of indirect assay. Among G-protein-coupled receptors are muscarinic acetylcholine receptors (mAChR), adrenergic receptors, serotonin receptors, dopamine receptors, angiotensin receptors, adenosine receptors, bradykinin receptors, metabotropic excitatory

amino acid receptors and the like. Cells expressing such G-protein-coupled receptors may exhibit increased cytoplasmic calcium levels as a result of contribution from both intracellular stores and via activation of ion channels, in which case it may be desirable although not necessary to conduct such assays in calcium-free buffer, optionally supplemented with a chelating agent such as EGTA, to distinguish fluorescence response resulting from calcium release from internal stores. Another type of indirect assay involves determining the activity of receptors which, when activated, result in a change in the level of intracellular cyclic nucleotides, e.g., cAMP, cGMP. For example, activation of some dopamine, serotonin, metabotropic glutamate receptors and muscarinic acetylcholine receptors results in a decrease in the cAMP or cGMP levels of the cytoplasm.

Furthermore, there are cyclic nucleotide-gated ion channels, e.g., rod photoreceptor cell channels and olfactory neuron channels [see, Altenhofen, W. et al. (1991) Proc. Natl. Acad. Sci U.S.A. 88:9868-9872 and Dhallan et al. (1990) Nature 347:184-187] that are permeable to cations upon activation by binding of cAMP or cGMP. A change in cytoplasmic ion levels caused by a change in the amount of cyclic nucleotide activation of photo-receptor or olfactory neuron channels is used to determine function of receptors that cause a change in cAMP or cGMP levels when activated. In cases where activation of the receptor results in a decrease in cyclic nucleotide levels, it may be preferable to expose the cells to agents that increase intracellular cyclic nucleotide levels, e.g., forskolin, prior to adding a receptor-activating compound to the cells in the assay. Cell for this type of assay can be made by co-transfection of a host cell with DNA encoding a cyclic nucleotide-gated ion channel and a DNA encoding a receptor (e.g., certain metabotropic glutamate receptors, muscarinic acetylcholine receptors, dopamine receptors, serotonin receptors and the like, which, when activated, causes a change in cyclic nucleotide levels in the cytoplasm.

Any cell expressing a receptor protein which is capable, upon activation, of directly increasing the intracellular concentration of calcium, such as by opening gated calcium channels, or indirectly affecting the concentration of intracellular calcium as by causing initiation of a reaction which utilizes Ca<2+> as a second messenger (e.g., G-protein-coupled receptors), may form the basis of an assay. Cells endogenously expressing such receptors or ion channels and cells which may be transfected with a suitable vector encoding one or more such cell surface proteins are known to those of skill in the art or may be identified by those of skill in the art. Although essentially any cell which expresses endogenous ion channel and/or receptor activity may be used, it is preferred to use cells transformed or transfected with heterologous DNAs encoding such ion channels and/or receptors so as to express predominantly a single type of ion channel or receptor. Many cells that may be genetically engineered to express a heterologous cell surface protein are known. Such cells include, but are not limited to, baby hamster kidney (BHK) cells (ATCC No. CCL10), mouse L cells (ATCC No. CCLI.3), DG44 cells [see, Chasin (1986) Cell. Molec. Genet. 12:555] human

embryonic kidney (HEK) cells (ATCC No. CRL1573), Chinese hamster ovary (CHO) cells (ATCC Nos. CRL9618, CCL61, CRL9096), PC12 cells (ATCC No. CRL1721) and COS-7 cells (ATCC No. CRL1651). Preferred cells for heterologous cell surface protein expression are those that can be readily and efficiently transfected. Preferred cells include HEK 293 cells, such as those described in U.S. Pat. No. 5,024,939.

Any compound which is known to activate ion channels or receptors of interest may be used to initiate an assay. Choosing an appropriate ion channel- or receptor-activating reagent depending on the ion channel or receptor of interest is within the skill of the art. Direct depolarization of the cell membrane to determine calcium channel activity may be accomplished by adding a potassium salt solution having a concentration of potassium ions such that the final concentration of potassium ions in the cell-containing well is in the range of about 50-150 mM (e.g., 50 mM KCl). With respect to ligand-gated receptors and ligand-gated ion channels, ligands are known which have affinity for and activate such receptors. For example, nicotinic acetyloholine receptors are known to be activated by nicotine or acetylcholine; similarly, muscarinic acetylcholine receptors may be activated by addition of muscarine or carbamylcholine.

Agonist assays may be carried out on cells known to possess ion channels and/or receptors to determine what effect, if any, a compound has on activation or potentiation of ion channels or receptors of interest. Agonist assays also may be carried out using a reagent known to possess ion channel- or receptor-activating capacity to determine whether a cell expresses the respective functional ion channel or receptor of interest.

Contacting a functional receptor or ion channel with agonist typically activates a transient reaction; and prolonged exposure to an agonist may desensitize the receptor or ion channel to subsequent activation. Thus, in general, assays for determining ion channel or receptor function should be initiated by addition of agonist (i.e., in a reagent solution used to initiate the reaction). The potency of a compound having agonist activity is determined by the detected change in some observable in the cells (typically an increase, although activation of certain receptors causes a decrease) as compared to the level of the observable in either the same cell, or substantially identical cell, which is treated substantially identically except that reagent lacking the agonist (i.e., control) is added to the well. Where an agonist assay is performed to test whether or not a cell expresses the functional receptor or ion channel of interest, known agonist is added to test-cell-containing wells and to wells containing control cells (substantially identical cell that lacks the specific receptors or ion channels) and the levels of observable are compared. Depending on the assay, cells lacking the ion channel and/or receptor of interest should exhibit substantially no increase in observable in response to the known agonist. A substantially identical cell may be derived from the same cells from which recombinant cells are prepared but which have not been modified by introduction of

heterologous DNA. Alternatively, it may be a cell in which the specific receptors or ion channels are removed. Any statistically or otherwise significant difference in the level of observable indicates that the test compound has in some manner altered the activity of the specific receptor or ion channel or that the test cell possesses the specific functional receptor or ion channel.

In an example of drug screening assays for identifying compounds which have the ability to modulate ion channels or receptors of interest, individual wells (or duplicate wells, etc.) contain a distinct cell type, or distinct recombinant cell line expressing a homogeneous population of a receptor or ion channel of interest, so that the compound having unidentified activity may be screened to determine whether it possesses modulatory activity with respect to one or more of a variety of functional ion channels or receptors. It is also contemplated that each of the individual wells may contain the same cell type so that multiple compounds (obtained from different reagent sources in the apparatus or contained within different wells) can be screened and compared for modulating activity with respect to one particular receptor or ion channel type.

Antagonist assays, including drug screening assays, may be carried out by incubating cells having functional ion channels and/or receptors in the presence and absence of one or more compounds, added to the solution bathing the cells in the respective wells of the microtiter plate for an amount of time sufficient (to the extent that the compound has affinity for the ion channel and/or receptor of interest) for the compound(s) to bind to the receptors and/or ion channels, then activating the ion channels or receptors by addition of known agonist, and measuring the level of observable in the cells as compared to the level of observable in either the same cell, or substantially identical cell, in the absence of the putative antagonist.

The assays are thus useful for rapidly screening compounds to identify those that modulate any receptor or ion channel in a cell. In particular, assays can be used to test functional ligand-receptor or ligand-ion channel interactions for cell receptors including ligand-gated ion channels, voltage-gated ion channels, G-protein-coupled receptors and growth factor receptors.

Those of ordinary skill in the art will recognize that assays may encompass measuring a detectable change of a solution as a consequence of a cellular event which allows a compound, capable of differential characteristics, to change its characteristics in response to the cellular event. By selecting a particular compound which is capable of differential characteristics upon the occurrence of a cellular event, various assays may be performed. For example, assays for determining the capacity of a compound to induce cell injury or cell death may be carried out by loading the cells with a pH-sensitive fluorescent indicator such as

BCECF (Molecular Probes, Inc., Eugene, Oreg. 97402, Catalog #B1150) and measuring cell injury or cell death as a function of changing fluorescence over time.

In a further example of useful assays, the function of receptors whose activation results in a change in the cyclic nucleotide levels of the cytoplasm may be directly determined in assays of cells that express such receptors and that have been injected with a fluorescent compound that changes fluorescence upon binding cAMP. The fluorescent compound comprises cAMP-dependent-protein kinase in which the catalytic and regulatory subunits are each labelled with a different fluorescent-dye [Adams. et al. (1991) Nature 349:694-697]. When cAMP binds to the regulatory subunits, the fluorescence emission spectrum changes; this change can be used as an indication of a change in cAMP concentration.

The function of certain neurotransmitter transporters which are present at the synaptic cleft at the junction between two neurons may be determined by the development of fluorescence in the cytoplasm of such neurons when conjugates of an amine acid and fluorescent indicator (wherein the fluorescent indicator of the conjugate is an acetoxymethyl ester derivative e.g., 5-(aminoacetamido)fluorescein; Molecular Probes, Catalog #A1363) are transported by the neurotransmitter transporter into the cytoplasm of the cell where the ester group is cleaved by esterase activity and the conjugate becomes fluorescent.

In practicing an assay of this type, a reporter gene construct is inserted into an eukaryotic cell to produce a recombinant cell which has present on its surface a cell surface protein of a specific type. The cell surface receptor may be endogenously expressed or it may be expressed from a heterologous gene that has been introduced into the cell. Methods for introducing heterologous DNA into eukaryotic cells are-well known in the art and any such method may be used. In addition, DNA encoding various cell surface proteins is known to those of skill in the art or it may be cloned by any method known to those of skill in the art.

The recombinant cell is contacted with a test compound and the level of reporter gene expression is measured. The contacting may be effected in any vehicle and the testing may be by any means using any protocols, such as serial dilution, for assessing specific molecular interactions known to those of skill in the art. After contacting the recombinant cell for a sufficient time to effect any interactions, the level of gene expression is measured. The amount of time to effect such interactions may be empirically determined, such as by running a time course and measuring the level of transcription as a function of time. The amount of transcription may be measured using any method known to those of skill in the art to be suitable. For example, specific mRNA expression may be detected using Northern blots or specific protein product may be identified by a characteristic stain. The amount of transcription is then compared to the amount of transcription in either the same cell in the absence of the test. compound or it may be compared with the amount of transcription in a substantially identical cell that lacks the specific receptors. A substantially identical cell may

be derived from the same cells from which the recombinant cell was prepared but which had not been modified by introduction of heterologous DNA. Alternatively, it may be a cell in which the specific receptors are removed. Any statistically or otherwise significant difference in the amount of transcription indicates that the test compound has in some manner altered the activity of the specific receptor.

If the test compound does not appear to enhance, activate or induce the activity of the cell surface protein, the assay may be repeated and modified by the introduction of a step in which the recombinant cell is first tested for the ability of a known agonist or activator of the specific receptor to activate transcription if the transcription is induced, the test compound is then assayed for its ability to inhibit, block or otherwise affect the activity of the agonist.

The transcription based assay is useful for identifying compounds that interact with any cell surface protein whose activity ultimately alters gene expression. In particular, the assays can be used to test functional ligand-receptor or ligand-ion channel interactions for a number of categories of cell surface-localized receptors, including: . ligand-gated ion channels and voltage-gated ion channels, and G protein-coupled receptors.

Any transfectable cell that can express the desired cell surface protein in a manner such the protein functions to intracellularly transduce an extracellular signal may be used. The cells may be selected such that they endogenously express the cell surface protein or may be genetically engineered to do so. Many such cells are known to those of skill in the art. Such cells include, but are not limited to Ltk< - > cells, PC12 cells and COS-7 cells.

The preparation of cells which express a receptor or ion channel and a reporter gene expression construct, and which are useful for .testing compounds to assess their activities, is exemplified in the Examples provided herewith by reference to mammalian Ltk< - > and COS-7 cell lines, which express the Type I human muscarinic (HM1) receptor and which are transformed with either a c-fos promoter-CAT reporter gene expression construct or a c-fos promoter-luciferase reporter gene expression construct.

Any cell surface protein that is known to those of skill in the art or that may be identified by those of skill in the art may used in the assay. The cell surface protein may endogenously expressed on the selected cell or it may be expressed from cloned DNA. Exemplary cell surface proteins include, but are not limited to, cell surface receptors and ion channels. Cell surface receptors include, but are not limited to, muscarinic receptors (e.g.,, human M2 (GenBank accession #M16404); rat M3 (GenBank accession #M16407); human M4 (GenBank accession #M16405); human M5 (Bonner et al. (1988) Neuron 1:403-410); and the like); neuronal nicotinic acetylcholine receptors (e.g., the alpha 2, alpha 3 and beta 2 subtypes disclosed in U.S. Ser. No. 504,455 (filed Apr. 3, 1990), hereby expressly incorporated by reference herein in its entirety); the rat alpha 2 subunit (Wada et al. (1988) Science 240:330-334); the rat alpha 3 subunit (Boulter et al. (1986) Nature 319:368-374); the

rat alpha 4 subunit (Goldman et al. (1987) cell 48:965-973); the rat alpha 5 subunit (Boulter et al. (1990) J. Biol. Chem. 265:4472-4482); the rat beta 2 subunit (Deneris et al. (1988) Neuron 1:45-54); the rat beta 3 subunit (Deneris et al. (1989) J. Biol. Chem. 264: 6268-6272); the rat beta 4 subunit (Duvoisin et al. (1989) Neuron 3:487-496); combinations of the rat alpha subunits, beta subunits and alpha and beta subunits; GABA receptors (e.g., the bovine alpha 1 and beta 1 subunits (Schofield et al. (1987) Nature 328:221-227); the bovine alpha 2 and alpha 3 subunits (Levitan et al. (1988) Nature 335:76-79); the gamma -subunit (Pritchett et al. (1989) Nature 338:582-585); the beta 2 and beta 3 subunits (Ymer et alo (1989) EMBO J. 8:1665-1670); the delta subunit (Shivers, B.D. (1989) Neuron 3:327-337); and the like); glutamate receptors (e.g., receptor isolated from rat brain (Hollmann et al. (1989) Nature 342:643-648); and the like); adrenergic receptors (e.g., human beta 1 (Frielle et al. (1987) Proc. Natl. Acad. Sci. 84.:7920-7924); human alpha 2 (Kobilka et al. (1987) Science 238:650-656); hamster beta 2 (Dixon et al. (1986) Nature 321:75-79); and the like); dopamine receptors (e.g., human D2 (Stormann et al. (1990) Molec. Pharm.37:1-6); rat (Bunzow et al. (1988) Nature 336:783-787); and the like); NGF receptors (e.g., human NGF receptors (Johnson et al. (1986) Cell 47:545-554); and the like); serotonin receptors (e.g., human 5HT1a (Kobilka et al. (1987) Nature 329:75-79); rat 5HT2 (Julius et al. (1990) PNAS 87:928-932); rat 5HTlc (Julius et al. (1988) Science 241:558-564); and the like).

Reporter gene constructs are prepared by operatively linking a reporter gene with at least one transcriptional regulatory element. If only one transcriptional regulatory element is included it must be a regulatable promoter, At least oneof the selected transcriptional regulatory elements must be indirectly or directly regulated by the activity of the selected cell-surface receptor whereby activity of the receptor can be monitored via transcription of the reporter genes.

The construct may contain additional transcriptional regulatory elements, such as a FIRE sequence, or other sequence, that is not necessarily regulated by the cell surface protein, but is selected for its ability to reduce background level transcription or to amplify the transduced signal and to thereby increase the sensitivity and reliability of the assay.

Many reporter genes and transcriptional regulatory elements are known to those of skill in the art and others may be identified or synthesized by methods known to those of skill in the art.

A reporter gene includes any gene that expresses a detectable gene product, which may be RNA or protein. Preferred reporter genes are those that are readily detectable. The reporter gene may also be included in the construct in the form of a fusion gene with a gene that includes desired transcriptional regulatory sequences or exhibits other desirable properties.

Examples of reporter genes include, but are not limited to CAT (chloramphenicol acetyl transferase) (Alton and Vapnek (1979), Nature 282: 864-869) luciferase, and other

enzyme detection systems, such as beta-galactosidase; firefly luciferase (deWet et al. (1987), Mol. Cell. Biol. 7:725-737); bacterial luciferase (Engebrecht and Silverman (1984), PNAS 1: 4154-4158; Baldwin et al. (1984), Biochemistry 23: 3663-3667); alkaline phosphatase (Toh et al. (1989) Eur. J. Biochem. 182: 231-238, Hall et al. (1983) J. Mol. Appl. Gen. 2: 101).

Transcriptional control elements include, but are not limited to, promoters, enhancers, and repressor and activator binding sites, Suitable transcriptional regulatory elements may be derived from the transcriptional regulatory regions of genes whose expression is rapidly induced, generally within minutes, of contact between the cell surface protein and the effector protein that modulates the activity of the cell surface protein. Examples of such genes include, but are not limited to, the immediate early genes (see, Sheng et al. (1990) Neuron 4: 477-485), such as c-fos, Immediate early genes are genes that are rapidly induced upon binding of a ligand to a cell surface protein. The transcriptional control elements that are preferred for use in the gene constructs include transcriptional control elements from immediate early genes, elements derived from other genes that exhibit some or all of the characteristics of the immediate early genes, or synthetic elements that are constructed such that genes in operative linkage therewith exhibit such characteristics. The characteristics of preferred genes from which the transcriptional control elements are derived include, but are not limited to, low or undetectable expression in quiescent cells, rapid induction at the transcriptional level within minutes of extracellular simulation, induction that is transient and independent of new protein synthesis, subsequent shut-off of transcription requires new protein synthesis, and mRNAs transcribed from these genes have a short half-life. It is not necessary for all of these properties to be present.

IV. Pharmaceutical Compositions

In another aspect, the present invention provides pharmaceutically acceptable compositions which comprise a therapeutically-effective amount of one or more of the compounds described above, formulated together with one or more pharmaceutically acceptable carriers (additives) and/or diluents. As described in detail below, the pharmaceutical compositions of the present invention may be specially formulated for administration in solid or liquid form, including those adapted for the following: (1) oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), tablets, boluses, powders, granules, pastes for application to the tongue; (2) parenteral administration, for example, by subcutaneous, intramuscular or intravenous injection as, for example, a sterile solution or suspension; (3) topical application, for example, as a cream, ointment or spray applied to the skin; or (4) intravaginally or intrarectally, for example, as a pessary, cream or foam.

The phrase "therapeutically-effective amount" as used herein means that amount of a compound, material, or composition comprising a compound of the present invention which is effective for producing some desired therapeutic effect in at least a sub-population of cells in an animal at a reasonable benefit/risk ratio applicable to any medical treatment.

The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

The phrase "pharmaceutically-acceptable carrier" as used herein means a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting the subject compound from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient. Some examples of materials which can serve as pharmaceutically-acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) phosphate buffer solutions; and (21) other non-toxic compatible substances employed in pharmaceutical formulations.

As set out above, certain embodiments of the present compounds may contain a basic functional group, such as amino or alkylamino, and are, thus, capable of forming pharmaceutically-acceptable salts with pharmaceutically-acceptable acids. The term "pharmaceutically-acceptable salts" in this respect, refers to the relatively non-toxic, inorganic and organic acid addition salts of compounds of the present invention. These salts can be prepared *in situ* during the final isolation and purification of the compounds of the invention, or by separately reacting a purified compound of the invention in its free base form with a suitable organic or inorganic acid, and isolating the salt thus formed. Representative salts include the hydrobromide, hydrochloride, sulfate, bisulfate, phosphate, nitrate, acetate, valerate, oleate, palmitate, stearate, laurate, benzoate, lactate, phosphate, tosylate, citrate, maleate, fumarate, succinate, tartrate, napthylate, mesylate, glucoheptonate, lactobionate, and

laurylsulphonate salts and the like. (See, for example, Berge et al. (1977) "Pharmaceutical Salts", *J. Pharm. Sci.* 66:1-19)

The pharmaceutically acceptable salts of the subject compounds include the conventional nontoxic salts or quaternary ammonium salts of the compounds, e.g., from non-toxic organic or inorganic acids. For example, such conventional nontoxic salts include those derived from inorganic acids such as hydrochloride, hydrobromic, sulfuric, sulfamic, phosphoric, nitric, and the like; and the salts prepared from organic acids such as acetic, propionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, palmitic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicyclic, sulfanilic, 2-acetoxybenzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isothionic, and the like.

In other cases, the compounds of the present invention may contain one or more acidic functional groups and, thus, are capable of forming pharmaceutically-acceptable salts with pharmaceutically-acceptable bases. The term "pharmaceutically-acceptable salts" in these instances refers to the relatively non-toxic, inorganic and organic base addition salts of compounds of the present invention. These salts can likewise be prepared *in situ* during the final isolation and purification of the compounds, or by separately reacting the purified compound in its free acid form with a suitable base, such as the hydroxide, carbonate or bicarbonate of a pharmaceutically-acceptable metal cation, with ammonia, or with a pharmaceutically-acceptable organic primary, secondary or tertiary amine. Representative alkali or alkaline earth salts include the lithium, sodium, potassium, calcium, magnesium, and aluminum salts and the like. Representative organic amines useful for the formation of base addition salts include ethylamine, diethylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine and the like. (See, for example, Berge et al., *supra*)

Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions.

Examples of pharmaceutically-acceptable antioxidants include: (1) water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

Administration of the adenosine antagonists and agonists for use in the method of this invention can be via any of the accepted modes of administration. These methods include, but are not limited to, oral, parenteral, transdermal, intraarticular and otherwise systemic administration. Oral administration is preferred. The compounds are administered in a

therapeutically effective amount either alone or in combination with a suitable pharmaceutically acceptable carrier or excipient.

Depending on the intended mode of administration, the adenosine antagonist or agonist of choice may be incorporated in any pharmaceutically acceptable dosage form, such as, for example, tablets, transdermal patches, pills, capsules, powders, liquids, suspensions, emulsions, aerosols or the like, preferably in unit dosage forms suitable for single administration of precise dosages, or sustained release dosage forms for continuous controlled administration. Preferably the dosage form will include a pharmaceutically acceptable excipient and, in addition, may contain other medicinal agents, pharmaceutical agents, carriers, adjuvants, and the like.

For solid dosage forms, non-toxic carriers include but are not limited to, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, the polyalkylene glycols, talcum, cellulose, glucose, sucrose and magnesium carbonate. Liquid pharmaceutically administrable dosage forms can, for example, comprise a solution or suspension of an active adenosine agent and optional pharmaceutical adjuvants in a carrier, such as, for example, water, saline aqueous dextrose, glycerol, ethanol and the like, to thereby form a solution or suspension. If desired, the pharmaceutical composition to be administered may also contain minor amounts of non-toxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like. Typical examples of such auxiliary agents are sodium acetate, sorbitan monolaurate, triethanolamine, sodium acetate, triethanolamine oleate, etc. Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in the art; for example, see: Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pa., 16th Edition, 1980. The composition of the formulation to be administered will, in any event, contain a quantity of the active adenosine agent in an amount effective for treatment.

Parenteral administration is generally characterized by injection, either subcutaneously, intramuscularly or intravenously. Injectables can be prepared in conventional forms, either as liquid solutions or suspension, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol and the like. In addition, if desired, the injectable pharmaceutical compositions to be administered may also contain minor amounts of non-toxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like.

The amount of active adenosine antagonist or agonist administered will, of course, be dependent on the subject being treated, the severity and nature of the affliction, the manner of administration, the potency and pharmacodynamics of the particular agent and the judgement of the prescribing physician. However, the therapeutically effective dosage for use in this invention will generally be in the range from about 0.01 mu g/kg (body weight) to 5 mg/kg.

Formulations of the present invention include those suitable for oral, nasal, topical (including buccal and sublingual), rectal, vaginal and/or parenteral administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the host being treated, the particular mode of administration. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will generally be that amount of the compound which produces a therapeutic effect. Generally, out of one hundred per cent, this amount will range from about 1 per cent to about ninety-nine percent of active ingredient, preferably from about 5 per cent to about 70 per cent, most preferably from about 10 per cent to about 30 per cent.

Methods of preparing these formulations or compositions include the step of bringing into association a compound of the present invention with the carrier and, optionally, one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association a compound of the present invention with liquid carriers, or finely divided solid carriers, or both, and then, if necessary, shaping the product.

Formulations of the invention suitable for oral administration may be in the form of capsules, cachets, pills, tablets, lozenges (using a flavored basis, usually sucrose and acacia or tragacanth), powders, granules, or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or as an elixir or syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia) and/or as mouth washes and the like, each containing a predetermined amount of a compound of the present invention as an active ingredient. A compound of the present invention may also be administered as a bolus, electuary or paste.

In solid dosage forms of the invention for oral administration (capsules, tablets, pills, dragees, powders, granules and the like), the active ingredient is mixed with one or more pharmaceutically-acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or any of the following: (1) fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and/or silicic acid; (2) binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose and/or acacia; (3) humectants, such as glycerol; (4) disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; (5) solution retarding agents, such as paraffin; (6) absorption accelerators, such as quaternary ammonium compounds; (7) wetting agents, such as, for example, cetyl alcohol and glycerol monostearate; (8) absorbents, such as kaolin and bentonite clay; (9) lubricants, such a talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; and (10) coloring agents. In the case of capsules, tablets and pills, the pharmaceutical compositions

may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared using binder (for example, gelatin or hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (for example, sodium starch glycolate or cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

The tablets, and other solid dosage forms of the pharmaceutical compositions of the present invention, such as dragees, capsules, pills and granules, may optionally be scored or prepared with coatings and shells, such as enteric coatings and other coatings well known in the pharmaceutical-formulating art. They may also be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile, other polymer matrices, liposomes and/or microspheres. They may be sterilized by, for example, filtration through a bacteria-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved in sterile water, or some other sterile injectable medium immediately before use. These compositions may also optionally contain opacifying agents and may be of a composition that they release the active ingredient(s) only, or preferentially, in a certain portion of the gastrointestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes. The active ingredient can also be in micro-encapsulated form, if appropriate, with one or more of the above-described excipients.

Liquid dosage forms for oral administration of the compounds of the invention include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active ingredient, the liquid dosage forms may contain inert diluents commonly used in the art, such as, for example, water or other solvents, solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof.

Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming and preservative agents.

Suspensions, in addition to the active compounds, may contain suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters,

microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

Formulations of the pharmaceutical compositions of the invention for rectal or vaginal administration may be presented as a suppository, which may be prepared by mixing one or more compounds of the invention with one or more suitable nonirritating excipients or carriers comprising, for example, cocoa butter, polyethylene glycol, a suppository wax or a salicylate, and which is solid at room temperature, but liquid at body temperature and, therefore, will melt in the rectum or vaginal cavity and release the active compound.

Formulations of the present invention which are suitable for vaginal administration also include pessaries, tampons, creams, gels, pastes, foams or spray formulations containing such carriers as are known in the art to be appropriate.

Dosage forms for the topical or transdermal administration of a compound of this invention include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. The active compound may be mixed under sterile conditions with a pharmaceutically-acceptable carrier, and with any preservatives, buffers, or propellants which may be required.

The ointments, pastes, creams and gels may contain, in addition to an active compound of this invention, excipients, such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof.

Powders and sprays can contain, in addition to a compound of this invention, excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder, or mixtures of these substances. Sprays can additionally contain customary propellants, such as chlorofluorohydrocarbons and volatile unsubstituted hydrocarbons, such as butane and propane.

Transdermal patches have the added advantage of providing controlled delivery of a compound of the present invention to the body. Such dosage forms can be made by dissolving or dispersing the compound in the proper medium. Absorption enhancers can also be used to increase the flux of the compound across the skin. The rate of such flux can be controlled by either providing a rate controlling membrane or dispersing the compound in a polymer matrix or gel.

Ophthalmic formulations, eye ointments, powders, solutions and the like, are also contemplated as being within the scope of this invention.

Pharmaceutical compositions of this invention suitable for parenteral administration comprise one or more compounds of the invention in combination with one or more pharmaceutically-acceptable sterile isotonic aqueous or nonaqueous solutions, dispersions,

suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain antioxidants, buffers, bacteriostats, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents.

Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of the action of microorganisms upon the subject compounds may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

In some cases, in order to prolong the effect of a drug, it is desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material having poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally-administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle.

Injectable depot forms are made by forming microencapsule matrices of the subject compounds in biodegradable polymers such as polylactide-polyglycolide. Depending on the ratio of drug to polymer, and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissue.

When the compounds of the present invention are administered as pharmaceuticals, to humans and animals, they can be given per se or as a pharmaceutical composition containing, for example, 0.1 to 99.5% (more preferably, 0.5 to 90%) of active ingredient in combination with a pharmaceutically acceptable carrier.

The preparations of the present invention may be given orally, parenterally, topically, or rectally. They are of course given in forms suitable for each administration route. . For example, they are administered in tablets or capsule form, by injection, inhalation, eye lotion, ointment, suppository, etc. administration by injection, infusion or inhalation; topical by lotion or ointment; and rectal by suppositories. Oral administrations are preferred.

The phrases "parenteral administration" and "administered parenterally" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticulare, subcapsular, subarachnoid, intraspinal and intrasternal injection and infusion.

The phrases "systemic administration," "administered systemically," "peripheral administration" and "administered peripherally" as used herein mean the administration of a compound, drug or other material other than directly into the central nervous system, such that it enters the patient's system and, thus, is subject to metabolism and other like processes, for example, subcutaneous administration.

These compounds may be administered to humans and other animals for therapy by any suitable route of administration, including orally; nasally, as by, for example, a spray, rectally, intravaginally, parenterally, intracisternally and topically, as by powders, ointments or drops, including buccally and sublingually.

Regardless of the route of administration selected, the compounds of the present invention, which may be used in a suitable hydrated form, and/or the pharmaceutical compositions of the present invention, are formulated into pharmaceutically-acceptable dosage forms by conventional methods known to those of skill in the art.

Actual dosage levels of the active ingredients in the pharmaceutical compositions of this invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient.

The selected dosage level will depend upon a variety of factors including the activity of the particular compound of the present invention employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compound employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

A physician or veterinarian having ordinary skill in the art can readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, the physician or veterinarian could start doses of the compounds of the invention employed in the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved.

In general, a suitable daily dose of a compound of the invention will be that amount of the compound which is the lowest dose effective to produce a therapeutic effect. Such an effective dose will generally depend upon the factors described above. Generally, intravenous, intracerebroventricular and subcutaneous doses of the compounds of this invention for a patient, when used for the indicated analgesic effects, will range from about 0.0001 to about 100 mg per kilogram of body weight per day.

If desired, the effective daily dose of the active compound may be administered as two, three, four, five, six or more sub-doses administered separately at appropriate intervals throughout the day, optionally, in unit dosage forms.

While it is possible for a compound of the present invention to be administered alone, it is preferable to administer the compound as a pharmaceutical formulation (composition).

In another aspect, the present invention provides pharmaceutically acceptable compositions which comprise a therapeutically-effective amount of one or more of the subject co,pounds, as described above, formulated together with one or more pharmaceutically acceptable carriers (additives) and/or diluents. As described in detail below, the pharmaceutical compositions of the present invention may be specially formulated for administration in solid or liquid form, including those adapted for the following: (1) oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), tablets, boluses, powders, granules, pastes for application to the tongue; (2) parenteral administration, for example, by subcutaneous, intramuscular or intravenous injection as, for example, a sterile solution or suspension; (3) topical application, for example, as a cream, ointment or spray applied to the skin; or (4) intravaginally or intravectally, for example, as a pessary, cream or foam.

The compounds according to the invention may be formulated for administration in any convenient way for use in human or veterinary medicine, by analogy with other pharmaceuticals.

The term "treatment" is intended to encompass also prophylaxis, therapy and cure.

The patient receiving this treatment is any animal in need, including primates, in particular humans, and other mammals such as equines, cattle, swine and sheep; and poultry and pets in general.

The compound of the invention can be administered as such or in admixtures with pharmaceutically acceptable carriers and can also be administered in conjunction with antimicrobial agents such as penicillins, cephalosporins, aminoglycosides and glycopeptides. Conjunctive therapy, thus includes sequential, simultaneous and separate administration of the active compound in a way that the therapeutical effects of the first administered one is not entirely disappeared when the subsequent is administered.

The addition of the active compound of the invention to animal feed is preferably accomplished by preparing an appropriate feed premix containing the active compound in an effective amount and incorporating the premix into the complete ration.

Alternatively, an intermediate concentrate or feed supplement containing the active ingredient can be blended into the feed. The way in which such feed premixes and complete rations can be prepared and administered are described in reference books (such as "Applied Animal Nutrition", W.H. Freedman and CO., San Francisco, U.S.A., 1969 or "Livestock Feeds and Feeding" O and B books, Corvallis, Ore., U.S.A., 1977).

V. Combinatorial Libraries

In the current era of drug development, high throughput screening of thousands to millions of compounds plays a key role. High throughput screening generally incorporates automation and robotics to enable testing these thousands to millions of compounds in one or more bioassays in a relatively short period of time. This high capacity screening technique requires enormous amounts of "raw materials" having immense molecular diversity to fill available capacity. Accordingly, combinatorial chemistry will play a significant role in meeting this demand for new molecules for screening. Once "leads" are identified using high throughput screening techniques, combinatorial chemistry will be advantageously used to optimize these initial leads (which analogs/variants will be tested in the same high throughput screening assay(s) that identified the initial lead).

A combinatorial library for the purposes of the present invention is a mixture of chemically-related compounds which may be screened together for a desired property; said libraries may be in solution or covalently linked to a solid support. The preparation of many related compounds in a single reaction greatly reduces and simplifies the number of screening processes which need to be carried out. Screening for the appropriate biological, pharmaceutical, agrochemical or physical property may be done by conventional methods.

Diversity in a library can be created at a variety of different levels. For instance, the substrate aryl groups used in a combinatorial approach can be diverse in terms of the core aryl

moiety, e.g., a variegation in terms of the ring structure, and/or can be varied with respect to the other substituents.

A variety of techniques are available in the art for generating combinatorial libraries of small organic molecules. See, for example, Blondelle et al. (1995) Trends Anal. Chem. 14:83; the Affymax U.S. Patents 5,359,115 and 5,362,899: the Ellman U.S. Patent 5,288,514: the Still et al. PCT publication WO 94/08051; Chen et al. (1994) JACS 116:2661: Kerr et al. (1993) JACS 115:252; PCT publications WO92/10092, WO93/09668 and WO91/07087; and the Lerner et al. PCT publication WO93/20242). Accordingly, a variety of libraries on the order of about 16 to 1,000,000 or more diversomers can be synthesized and screened for a particular activity or property.

In an exemplary embodiment, a library of substituted diversomers can be synthesized using the subject reactions adapted to the techniques described in the Still et al. PCT publication WO 94/08051, e.g., being linked to a polymer bead by a hydrolyzable or photolyzable group, e.g., located at one of the positions of substrate. According to the Still et al. technique, the library is synthesized on a set of beads, each bead including a set of tags identifying the particular diversomer on that bead. In one embodiment, which is particularly suitable for discovering enzyme inhibitors, the beads can be dispersed on the surface of a permeable membrane, and the diversomers released from the beads by lysis of the bead linker. The diversomer from each bead will diffuse across the membrane to an assay zone, where it will interact with an enzyme assay. Detailed descriptions of a number of combinatorial methodologies are provided below.

A) Direct Characterization

A growing trend in the field of combinatorial chemistry is to exploit the sensitivity of techniques such as mass spectrometry (MS), e.g., which can be used to characterize sub-femtomolar amounts of a compound, and to directly determine the chemical constitution of a compound selected from a combinatorial library. For instance, where the library is provided on an insoluble support matrix, discrete populations of compounds can be first released from the support and characterized by MS. In other embodiments, as part of the MS sample preparation technique, such MS techniques as MALDI can be used to release a compound from the matrix, particularly where a labile bond is used originally to tether the compound to the matrix. For instance, a bead selected from a library can be irradiated in a MALDI step in order to release the diversomer from the matrix, and ionize the diversomer for MS analysis.

B) Multipin Synthesis

The libraries of the subject method can take the multipin library format. Briefly, Geysen and co-workers (Geysen et al. (1984) PNAS 81:3998-4002) introduced a method for generating compound libraries by a parallel synthesis on polyacrylic acid-grated polyethylene pins arrayed in the microtitre plate format. The Geysen technique can be used to synthesize and screen thousands of compounds per week using the multipin method, and the tethered compounds may be reused in many assays. Appropriate linker moieties can also been appended to the pins so that the compounds may be cleaved from the supports after synthesis for assessment of purity and further evaluation (c.f., Bray et al. (1990) Tetrahedron Lett 31:5811-5814; Valerio et al. (1991) Anal Biochem 197:168-177; Bray et al. (1991) Tetrahedron Lett 32:6163-6166).

C) Divide-Couple-Recombine

In yet another embodiment, a variegated library of compounds can be provided on a set of beads utilizing the strategy of divide-couple-recombine (see, e.g., Houghten (1985) PNAS 82:5131-5135; and U.S. Patents 4,631,211; 5,440,016; 5,480,971). Briefly, as the name implies, at each synthesis step where degeneracy is introduced into the library, the beads are divided into separate groups equal to the number of different substituents to be added at a particular position in the library, the different substituents coupled in separate reactions, and the beads recombined into one pool for the next iteration.

In one embodiment, the divide-couple-recombine strategy can be carried out using an analogous approach to the so-called "tea bag" method first developed by Houghten, where compound synthesis occurs on resin sealed inside porous polypropylene bags (Houghten et al. (1986) PNAS 82:5131-5135). Substituents are coupled to the compound-bearing resins by placing the bags in appropriate reaction solutions, while all common steps such as resin washing and deprotection are performed simultaneously in one reaction vessel. At the end of the synthesis, each bag contains a single compound.

D) Combinatorial Libraries by Light-Directed, Spatially Addressable Parallel Chemical Synthesis

A scheme of combinatorial synthesis in which the identity of a compound is given by its locations on a synthesis substrate is termed a spatially-addressable synthesis. In one embodiment, the combinatorial process is carried out by controlling the addition of a

chemical reagent to specific locations on a solid support (Dower et al. (1991) Annu Rep Med Chem 26:271-280; Fodor, S.P.A. (1991) Science 251:767; Pirrung et al. (1992) U.S. Patent No. 5,143,854; Jacobs et al. (1994) Trends Biotechnol 12:19-26). The spatial resolution of photolithography affords miniaturization. This technique can be carried out through the use protection/deprotection reactions with photolabile protecting groups.

The key points of this technology are illustrated in Gallop et al. (1994) J Med Chem 37:1233-1251. A synthesis substrate is prepared for coupling through the covalent attachment of photolabile nitroveratryloxycarbonyl (NVOC) protected amino linkers or other photolabile linkers. Light is used to selectively activate a specified region of the synthesis support for coupling. Removal of the photolabile protecting groups by light (deprotection) results in activation of selected areas. After activation, the first of a set of amino acid analogs, each bearing a photolabile protecting group on the amino terminus, is exposed to the entire surface. Coupling only occurs in regions that were addressed by light in the preceding step. The reaction is stopped, the plates washed, and the substrate is again illuminated through a second mask, activating a different region for reaction with a second protected building block. The pattern of masks and the sequence of reactants define the products and their locations. Since this process utilizes photolithography techniques, the number of compounds that can be synthesized is limited only by the number of synthesis sites that can be addressed with appropriate resolution. The position of each compound is precisely known; hence, its interactions with other molecules can be directly assessed.

In a light-directed chemical synthesis, the products depend on the pattern of illumination and on the order of addition of reactants. By varying the lithographic patterns, many different sets of test compounds can be synthesized simultaneously; this characteristic leads to the generation of many different masking strategies.

E) Encoded Combinatorial Libraries

In yet another embodiment, the subject method utilizes a compound library provided with an encoded tagging system. A recent improvement in the identification of active compounds from combinatorial libraries employs chemical indexing systems using tags that uniquely encode the reaction steps a given bead has undergone and, by inference, the structure it carries. Conceptually, this approach mimics phage display libraries, where activity derives from expressed peptides, but the structures of the active peptides are deduced from the corresponding genomic DNA sequence. The first encoding of synthetic combinatorial libraries employed DNA as the code. A variety of other forms of encoding have been

reported, including encoding with sequenceable bio-oligomers (e.g., oligonucleotides and peptides), and binary encoding with additional non-sequenceable tags.

1) Tagging with sequenceable bio-oligomers

The principle of using oligonucleotides to encode combinatorial synthetic libraries was described in 1992 (Brenner et al. (1992) PNAS 89:5381-5383), and an example of such a library appeared the following year (Needles et al. (1993) PNAS 90:10700-10704). A combinatorial library of nominally 7^7 (= 823,543) peptides composed of all combinations of Arg, Gln, Phe, Lys, Val, d-Val and Thr (three-letter amino acid code), each of which was encoded by a specific dinucleotide (TA, TC, CT, AT, TT, CA and AC, respectively), was prepared by a series of alternating rounds of peptide and oligonucleotide synthesis on solid support. In this work, the amine linking functionality on the bead was specifically differentiated toward peptide or oligonucleotide synthesis by simultaneously preincubating the beads with reagents that generate protected OH groups for oligonucleotide synthesis and protected NH2 groups for peptide synthesis (here, in a ratio of 1:20). When complete, the tags each consisted of 69-mers, 14 units of which carried the code. The bead-bound library was incubated with a fluorescently labeled antibody, and beads containing bound antibody that fluoresced strongly were harvested by fluorescence-activated cell sorting (FACS). The DNA tags were amplified by PCR and sequenced, and the predicted peptides were synthesized. Following such techniques, compound libraries can be derived for use in the subject method, where the oligonucleotide sequence of the tag identifies the sequential combinatorial reactions that a particular bead underwent, and therefore provides the identity of the compound on the bead.

The use of oligonucleotide tags permits exquisitely sensitive tag analysis. Even so, the method requires careful choice of orthogonal sets of protecting groups required for alternating co-synthesis of the tag and the library member. Furthermore, the chemical lability of the tag, particularly the phosphate and sugar anomeric linkages, may limit the choice of reagents and conditions that can be employed for the synthesis of non-oligomeric libraries. In certain embodiments, the libraries employ linkers permitting selective detachment of the test compound library member for assay.

Peptides have also been employed as tagging molecules for combinatorial libraries. Two exemplary approaches are described in the art, both of which employ branched linkers to solid phase upon which coding and ligand strands are alternately elaborated. In the first approach (Kerr JM et al. (1993) J Am Chem Soc 115:2529-2531), orthogonality in

synthesis is achieved by employing acid-labile protection for the coding strand and base-labile protection for the compound strand.

In an alternative approach (Nikolaiev et al. (1993) Pept Res 6:161-170), branched linkers are employed so that the coding unit and the test compound can both be attached to the same functional group on the resin. In one embodiment, a cleavable linker can be placed between the branch point and the bead so that cleavage releases a molecule containing both code and the compound (Ptek et al. (1991) Tetrahedron Lett 32:3891-3894). In another embodiment, the cleavable linker can be placed so that the test compound can be selectively separated from the bead, leaving the code behind. This last construct is particularly valuable because it permits screening of the test compound without potential interference of the coding groups. Examples in the art of independent cleavage and sequencing of peptide library members and their corresponding tags has confirmed that the tags can accurately predict the peptide structure.

2) Non-sequenceable Tagging: Binary Encoding

An alternative form of encoding the test compound library employs a set of non-sequencable electrophoric tagging molecules that are used as a binary code (Ohlmeyer et al. (1993) PNAS 90:10922-10926). Exemplary tags are haloaromatic alkyl ethers that are detectable as their trimethylsilyl ethers at less than femtomolar levels by electron capture gas chromatography (ECGC). Variations in the length of the alkyl chain, as well as the nature and position of the aromatic halide substituents, permit the synthesis of at least 40 such tags, which in principle can encode 240 (e.g., upwards of 1012) different molecules. In the original report (Ohlmeyer et al., supra) the tags were bound to about 1% of the available amine groups of a peptide library via a photocleavable o-nitrobenzyl linker. This approach is convenient when preparing combinatorial libraries of peptide-like or other amine-containing molecules. A more versatile system has, however, been developed that permits encoding of essentially any combinatorial library. Here, the compound would be attached to the solid support via the photocleavable linker and the tag is attached through a catechol ether linker via carbene insertion into the bead matrix (Nestler et al. (1994) J Org Chem 59:4723-4724). This orthogonal attachment strategy permits the selective detachment of library members for assay in solution and subsequent decoding by ECGC after oxidative detachment of the tag sets.

Although several amide-linked libraries in the art employ binary encoding with the electrophoric tags attached to amine groups, attaching these tags directly to the bead matrix provides far greater versatility in the structures that can be prepared in encoded combinatorial

libraries. Attached in this way, the tags and their linker are nearly as unreactive as the bead matrix itself. Two binary-encoded combinatorial libraries have been reported where the electrophoric tags are attached directly to the solid phase (Ohlmeyer et al. (1995) PNAS 92:6027-6031) and provide guidance for generating the subject compound library. Both libraries were constructed using an orthogonal attachment strategy in which the library member was linked to the solid support by a photolabile linker and the tags were attached through a linker cleavable only by vigorous oxidation. Because the library members can be repetitively partially photoeluted from the solid support, library members can be utilized in multiple assays. Successive photoelution also permits a very high throughput iterative screening strategy: first, multiple beads are placed in 96-well microtiter plates; second, compounds are partially detached and transferred to assay plates; third, a metal binding assay identifies the active wells; fourth, the corresponding beads are rearrayed singly into new microtiter plates; fifth, single active compounds are identified; and sixth, the structures are decoded.

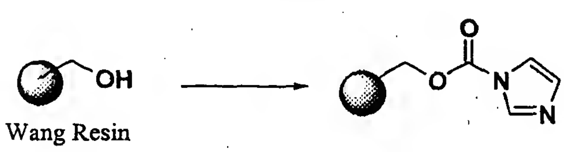
Exemplification

The invention now being generally described, it will be more readily understood by reference to the following examples, which are included merely for purposes of illustration of certain aspects and embodiments of the present invention, and are not intended to limit the invention.

Examples 1-7 and 14-18 exemplify the synthesis of a diarylthioether on a solid support. Examples 8-11 exemplify the synthesis of a diarylthioether in solution.

Example 1

Synthesis of resin 2

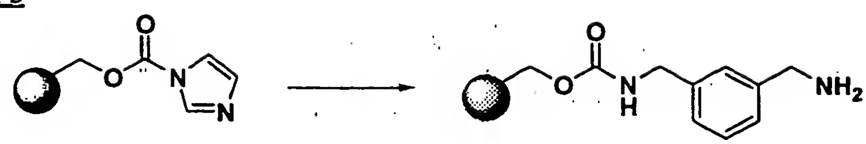


1 2

Wang resin (1, 50 g, 0.69 mmol/g) was treated with 1,1'-carbonyldiimidazole (CDI) in THF at room temperature for 18h, washed several times with THF and dried *in vacuo* to give **2**.

Example 2

Synthesis of 3

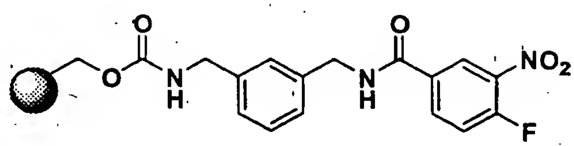


2 3

Resin **2** was coupled to m-xylenediamine (THF, rt, 2h) to give **3** according to the procedure outline by Hauske et al. (Tetrahedron Lett. **1995**, *36*, 1589-1592). The resin was washed several times with THF, methanol, and dichloromethane before being dried *in vacuo* to give resin **3**.

Example 3

Synthesis of 4

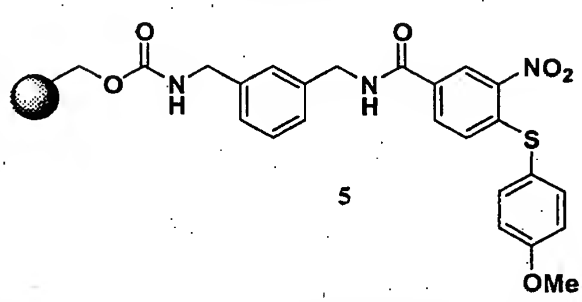


4

Resin **3** was allowed to react with 4-fluoro-3-nitrobenzoic acid (4.3 equiv.) in the presence of PyBOP (5 equiv.; PyBOP: bromo-tris-pyrrolidinophosphonium hexafluorophosphate) and N-methylmorpholine (NMM, 10 equiv) in DMF at room temperature for 3h, washed sequentially with DMF, methanol, and dichloromethane, and then dried *in vacuo* to give **4**.

Example 4

Synthesis of 5

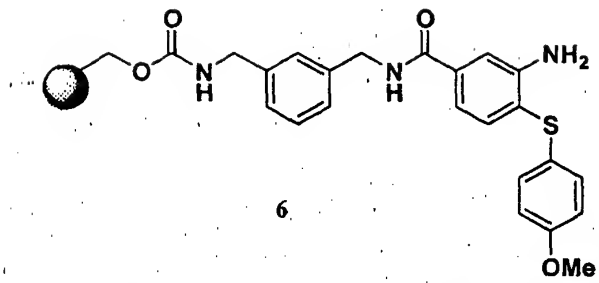


5

Resin **4** was allowed to react with 4-methoxythiophenol (5.0 equiv) in the presence of triethylamine (5 equiv) in DMF at room temperature for 5h, washed sequentially with DMF, methanol, and dichloromethane, and then dried *in vacuo* to give **5**.

Example 5

Synthesis of **6**

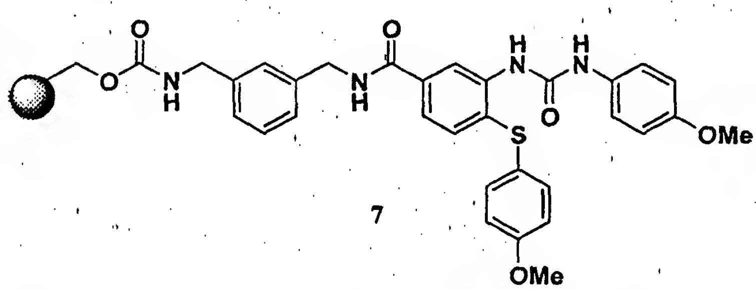


6

Resin **5** (3.0 g) was allowed to react with tin dichloride dihydrate (27 g) in DMF (60 mL) at room temperature for approximately 18 h. The resin was washed sequentially with DMF, methanol, and dichloromethane, and then dried *in vacuo* to give **6**.

Example 6

Synthesis of **7**

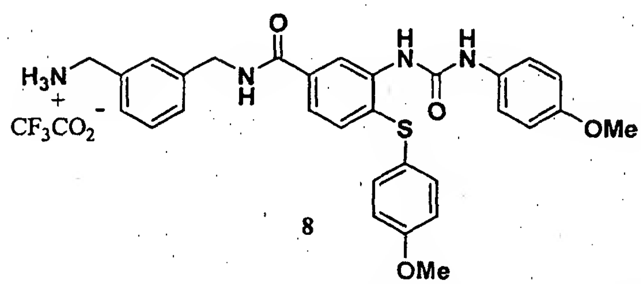


7

Resin **6** (3.0 g) was allowed to react with p-methoxyphenylisocyanate (10 equiv) and N,N-dimethyl-4-aminopyridine (10 mg) in dichloromethane (60 mL) at room temperature for approximately 18 h. The resin was washed sequentially with dichloromethane, methanol, and dichloromethane, and then dried *in vacuo* to give **7**.

Example 7

Synthesis of **8**

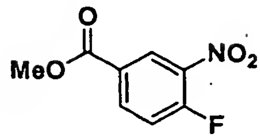


8

Resin **7** (3.0 g) was allowed to react with 50% trifluoroacetic acid in dichloromethane (60 mL) at room temperature for approximately 30 min. The resin was filtered. The filtrate was concentrated to a residue. The residue was purified by reverse-phase preparative HPLC using acetonitrile and water as eluant to give **8**.

Example 8

Synthesis of **9**



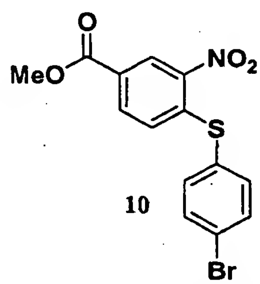
9

A round-bottom flask was charged with 4-fluoro-3-nitrobenzoic acid (5.55 g , 30 mmol) and methanol (30 mL). After cooling the mixture to 0° C, thionyl chloride (2.4 mL, 33 mmol) was slowly added. The resulting solution was heated at reflux overnight. The solution was allowed to cool to room temperature. During the cooling process precipitation occurred. The mixture was diluted with 50% methanol in water and then filtered. The residue was washed with 50% methanol in water (30 mL) and dried to give **9** (4.83 g, 81%) as a white solid.

Example 9

Synthesis of **10**

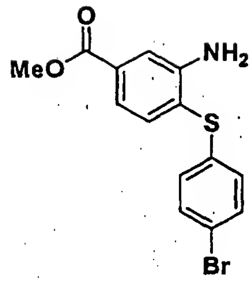
10



A round-bottom flask was charged with **9** (398 mg , 2.0 mmol), DMF (7 mL), 4-bromothiophenol (1.1 equiv.), and triethylamine (0.306 mL, 2.2 mmol). The reaction was stirred at room temperature for 2h and then diluted with water (100 mL). The mixture was extracted with ethyl acetate (2 times 100 mL). The extracts were combined, washed sequentially with water (50 mL) and brine (50 mL). The organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated to give **10** as a yellow oil.

Example 10

Synthesis of **11**

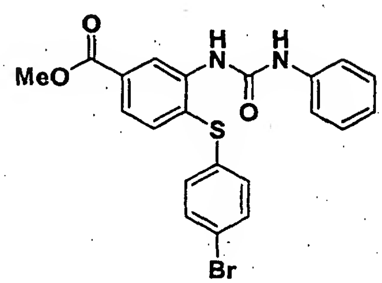


11

A round-bottom flask was charged with **10** (2.0 mmol), ethanol (8 mL), iron powder (2.5 equiv.), and concentrated hydrochloric acid (1.9 mL). The reaction was stirred at reflux for 1h, cool to room temperature, and diluted with water (20 mL). The mixture was neutralized with saturated sodium bicarbonate, extracted with ethyl acetate (3 times 100 mL). The extracts were combined, washed sequentially with water (50 mL) and brine (50 mL). The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated to give **11** as a yellow oil.

Example 11

Synthesis of **12**

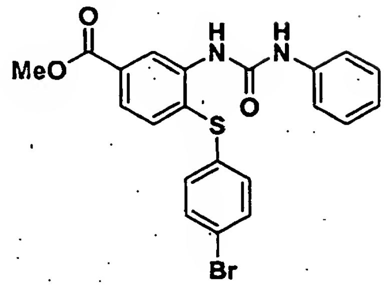


12

A round-bottom flask was charged with **11** (1.82 mmol), dichloromethane (40 mL), phenylisocyanate (1.1 equiv.), and N,N-dimethyl-4-aminopyridine (8 mg). The reaction was stirred at 40 °C for overnight, cool to room temperature, and diluted with water (20 mL). The mixture was extracted with ethyl acetate (3 times 100 mL). The extracts were combined, washed sequentially with water (50 mL) and brine (50 mL). The organic layer was dried over anhydrous sodium sulfate, filtered, and concentrated. The residue was purified by column chromatography to give **12** as a white solid.

Example 12

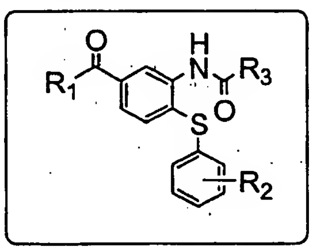
Activity Against Human Adenosine A_3 Receptors



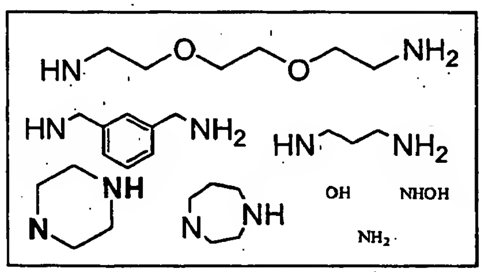
The adenosine (A_1, A_{2A}, and A_3) receptor binding capabilities of compounds described herein were determined according to the procedures outlined by Libert et al. (*Biochem. Comm.* **1992,** *187*, 919), Varani et al. (*Br. J. Pharmacol.* **1996,** *117*, 1693), and Olah et al. (*Mol. Pharmacol.* **1994,** *45*, 978). The compound depicted in this Example displayed a K_i of 2.7 μM against human adenosine A_3 receptors.

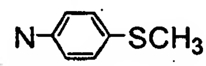
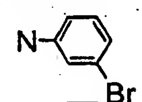
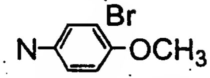
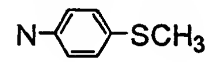
Example 13

Serotonin Assays



R_1=



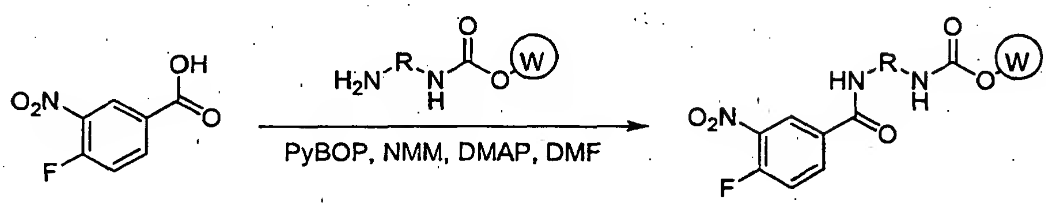
Assay	Concentration (μM)	% Inhibition	R_2	R_3
Serotonin Transporter	1	99	3,5 dimethyl	
5-HT_{1B}	1	93	4-OH	
5-HT_2	1	90	4-OCH_3	
5-HT_3	1	52	3-OCH_3	

The serotonin transporter and serotonin (5-HT_{1B}, 5-HT_2, and 5-HT_3) receptor binding capabilities of compounds depicted in this Example were determined according to the procedures outlined by Gu et al. (*J. Biol. Chem.* **1994,** *269*, 7124), Hoyer et al. (*Eur. J.*

Pharmacol. **1985**, *118*, 1), Leysen et al. (*Mol. Pharmacol.* **1982**, *21*, 301), and/or Pinkus et al. (*Eur. J. Pharmacol.* **1989**, *168*, 355).

Example 14

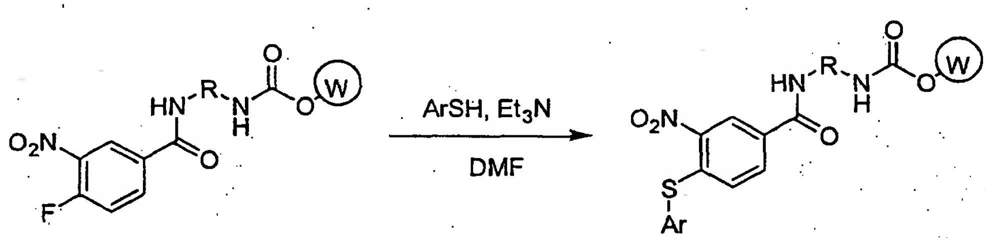
Synthesis of Resin-Bound 4-Fluoro-3-nitrobenzoic Acid



To a 250 mL round-bottom flask was added 4-fluoro-3-nitrobenzoic acid (3.24 g, 17.5 mmol), PyBOP (9.1 g, 17.5 mmol), DMF (100 mL) and NMM (3.85 mL, 35 mmol). The mixture was stirred for 10 min and then added to a fritted flask containing diamine resin (5.0 g, 3.5 mmol) and DMAP (43 mg, 0.35 mmoL). The mixture was agitated for 24 h and then filtered. The resin was rinsed with DMF (3x100 mL), MeOH (3x100 mL) and DCM (3x100 mL) and then dried under vacuum to give resin-bound 4-fluoro-3-nitrobenzoic acid.

Example 15

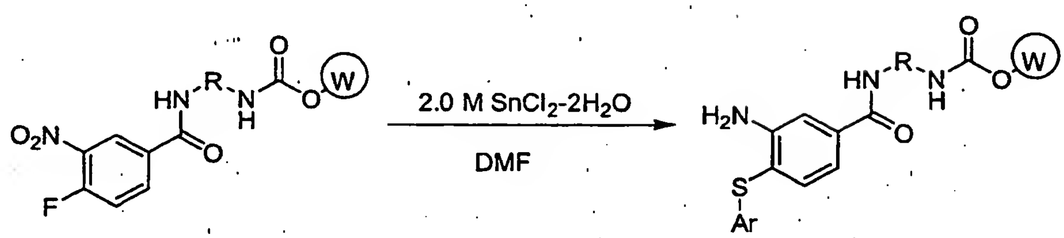
Synthesis of Resin-Bound 4-Arylthio-3-nitrobenzoic Acids



To a 20 mL fritted tube was added resin-bound 4-fluoro-3-nitrobenzoic acid (1.0 g, 0.7 mmol), DMF (10 mL), arylthiol (3.5 mmol) and triethylamine (0.488 mL, 3.5 mmol). The tube was agitated for 24 h and then filtered. The resin was rinsed with DMF (3x10 mL), MeOH (3x10 mL) and DCM (3x10 mL) and then dried under vacuum to give resin-bound 4-arylthio-3-nitrobenzoic acid.

Example 16

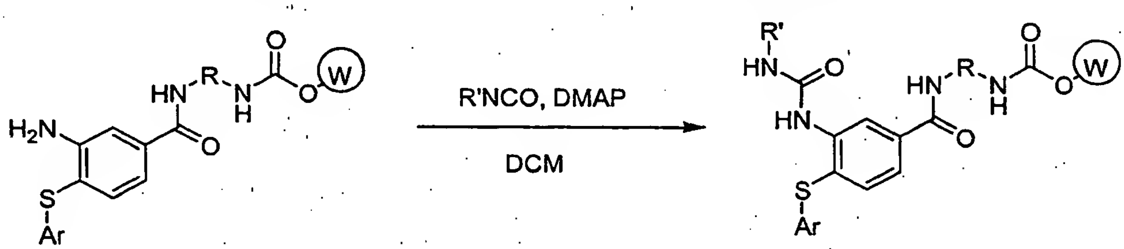
Synthesis of Resin-Bound 4-Arylthio-3-aminobenzoic Acids



To a 10 mL fritted tube was added resin-bound 4-arylthio-3-nitrobenzoic acid (0.5 g, 0.35 mmol) and a 2.0 M solution of tin (II) chloride dihydrate (3 mL) in DMF. The tube was agitated for 24 h and then filtered. The resin was rinsed with DMF (3x5 mL), MeOH (3x5 mL) and DCM (3x5 mL) and then dried under vacuum to give resin-bound 4-arylthio-3-aminobenzoic acid.

Example 17

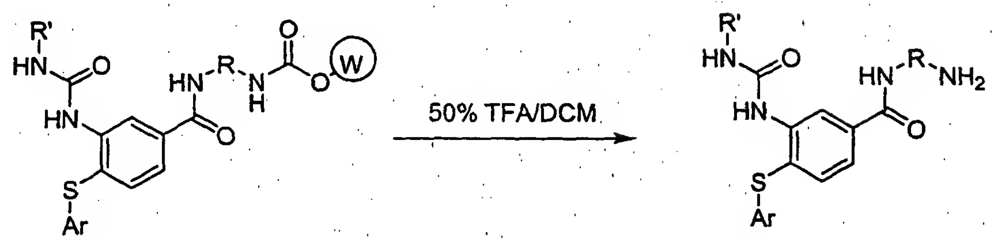
Synthesis of Resin-Bound 4-Arylthio-3-alkyl or acylureabenzoic Acids



To a 6 mL fritted tube was added resin-bound 4-arylthio-3-aminobenzoic acid (0.25 g, 0.18 mmol), DMAP (2 mg, 0.02 mmol), DCM (3 mL), and isocyanate (0.88 mmol). The tube was agitated for 24 h and then filtered. The resin was rinsed with DMF (3x5 mL), MeOH (3x5 mL) and DCM (3x5 mL) and then dried under vacuum to give resin-bound 4-arylthio-3-alkyl or acylureabenzoic acid.

Example 18

Synthesis of 4-Arylthio-3-alkyl or acylureabenzamides



To a 6 mL fritted tube was added resin-bound 4-arylthio-3-aminobenzoic acid (0.25 g, 0.18 mmol) and 1:1 TFA/DCM (3 mL). The tube was agitated for 1 h and then filtered. The resin was DCM (3x1 mL) and the solutions combined and concentrated *in vacuo*. The crude products were purified by preparatory HPLC (C-18, MeCN/H_2O 65:35) to give pure 4-arylthio-3-alkyl or acylureabenzamides (20-60% yield overall).

Equivalents

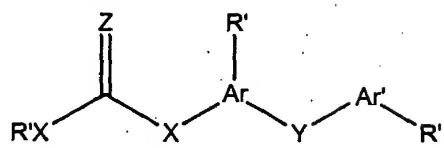
Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

Incorporation by Reference

All of the patents and publications cited herein are hereby incorporated by reference.

We claim:

1. The compound represented by general structure **1**:



1

wherein

Ar and Ar' are independently selected from the group consisting of aromatic and heteroaromatic moieties;

X represents independently for each occurrence O, S, Se, NR, PR or AsR;

Y represents O, S, Se, NR, PR or AsR;

Z represents O, S, Se, NR, PR or AsR;

R represents independently for each occurrence hydrogen, optionally substituted alkyl, aryl, or heteroaryl; or formyl, acyl, sulfonyl, $-Si(alkyl)_d(aryl)_g$, $-Sn(alkyl)_d(aryl)_g$, or $-(CH_2)_mR_8$;

R' represents independently for each occurrence halogen, alkyl, alkenyl, alkynyl, hydroxyl, alkoxyl, silyloxy, amino, nitro, sulfhydryl, alkylthio, imine, amide, phosphoryl, phosphonate, phosphine, carbonyl, carboxyl, carboxamide, anhydride, silyl, thioalkyl, alkylsulfonyl, arylsulfonyl, selenoalkyl, ketone, aldehyde, ester, heteroalkyl, nitrile, guanidine, amidine, acetal, ketal, amine oxide, aryl, heteroaryl, azide, aziridine, carbamate, epoxide, hydroxamic acid, imide, oxime, sulfonamide, thioamide, thiocarbamate, urea, thiourea, or $-(CH_2)_mR_8$;

Ar or Ar' may be unsubstituted beyond X and Y, or Y, respectively, i.e., R' may be absent, or they may be substituted with R' any number of times consistent with the limitations imposed by the rules of valence;

R_8 represents independently for each occurrence an optionally substituted aryl, cycloalkyl, cycloalkenyl, heterocycle or polycycle;

d and g are integers independently selected from the range 0 to 3 inclusive, whose sum is 3; and

m is an integer in the range 0 to 8 inclusive.

2. The compound of claim 1, wherein X represents NR.

3. The compound of claim 1, wherein X represents NH.

4. The compound of claim 1, wherein Y represents S.

5. The compound of claim 1, wherein Z represents O.

6. The compound of claim 1, wherein Ar represents phenyl.

7. The compound of claim 1, wherein there is a single instance of R' on Ar, and said instance of R' represents -C(O)NR_2.

8. The compound of claim 1, wherein X represents NR; and Y represents S.

9. The compound of claim 1, wherein X represents NR; and Z represents O.

10. The compound of claim 1, wherein X represents NR; and Ar represents phenyl.

11. The compound of claim 1, wherein X represents NR; and there is a single instance of R' on Ar, and said instance of R' represents -C(O)NR_2.

12. The compound of claim 1, wherein X represents NH; and Y represents S.

13. The compound of claim 1, wherein X represents NH; and Z represents O.

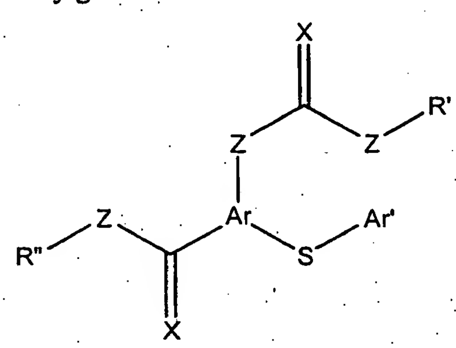
14. The compound of claim 1, wherein X represents NH; and Ar represents phenyl.

15. The compound of claim 1, wherein X represents NH; and there is a single instance of R' on Ar, and said instance of R' represents -C(O)NR_2.

16. The compound of claim 1, wherein Ar represents phenyl; X represents NR; Y represents S; Z represents O; and there is a single instance of R' on Ar, and said instance of R' represents -C(O)NR_2.

17. The compound of claim 1, wherein Ar represents phenyl; X represents NH; Y represents S; Z represents O; and there is a single instance of R' on Ar, and said instance of R' represents -C(O)NR_2.

18. The compound represented by general structure **2**:



2

wherein

Ar and Ar' are independently selected from the group consisting of optionally substituted aromatic and heteroaromatic moieties;

X represents independently for each occurrence O, S, Se, NR, PR or AsR;

Z represents independently for each occurrence O, S, Se, NR, PR or AsR;

R represents independently for each occurrence hydrogen, optionally substituted alkyl, aryl, or heteroaryl; or formyl, acyl, sulfonyl, -Si(alkyl)$_d$(aryl)$_g$, -Sn(alkyl)$_d$(aryl)$_g$, or -$(CH_2)_mR_8$;

R' and R" represent independently for each occurrence hydrogen, halogen, alkyl, alkenyl, alkynyl, hydroxyl, alkoxyl, silyloxy, amino, nitro, sulfhydryl, alkylthio, imine, amide, phosphoryl, phosphonate, phosphine, carbonyl, carboxyl, carboxamide, anhydride, silyl, thioalkyl, alkylsulfonyl, arylsulfonyl, selenoalkyl, ketone, aldehyde, ester, heteroalkyl, nitrile, guanidine, amidine, acetal, ketal, amine oxide, aryl, heteroaryl, azide, aziridine, carbamate, epoxide, hydroxamic acid, imide, oxime, sulfonamide, thioamide, thiocarbamate, urea, thiourea, or -$(CH_2)_mR_8$;

R_8 represents independently for each occurrence an optionally substituted aryl, cycloalkyl, cycloalkenyl, heterocycle or polycycle;

d and g are integers independently selected from the range 0 to 3 inclusive, whose sum is 3; and

m is an integer in the range 0 to 8 inclusive.

19. The compound of claim 18, wherein X represents O.

20. The compound of claim 18, wherein Z represents NR.

21. The compound of claim 18, wherein R' represents hydrogen, or optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl.

22. The compound of claim 18, wherein R" represents hydrogen, hydroxyl, or optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl; or aminoalkyl, amino(heteroalkyl); or ZR" taken together represents hydroxyl or a heterocycle comprising Z and a secondary amine.

23. The compound of claim 18, wherein Ar represents phenyl.

24. The compound of claim 18, wherein Ar' represents optionally substituted phenyl, pyridyl, or quinolinyl.

25. The compound of claim 18, wherein X represents O; and Z represents NR.

26. The compound of claim 18, wherein X represents O; and R' represents hydrogen, or optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl.

27. The compound of claim 18, wherein X represents O; and R" represents hydrogen, hydroxyl, or optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl; or aminoalkyl, or amino(heteroalkyl); or ZR" taken together represents hydroxyl or a heterocycle comprising Z and a secondary amine.

28. The compound of claim 18, wherein X represents O; and Ar represents phenyl.

29. The compound of claim 18, wherein X represents O; and Ar' represents optionally substituted phenyl, pyridyl, or quinolinyl.

30. The compound of claim 18, wherein Z represents NR; and R' represents hydrogen, or optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl.

31. The compound of claim 18, wherein Z represents NR; and R" represents hydrogen, hydroxyl, or optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl; or aminoalkyl, or amino(heteroalkyl); or ZR" taken together represents hydroxyl or a heterocycle comprising Z and a secondary amine.

32. The compound of claim 18, wherein Z represents NR; and Ar represents phenyl.

33. The compound of claim 18, wherein Z represents NR; and Ar' represents optionally substituted phenyl, pyridyl, or quinolinyl.

34. The compound of claim 18, wherein Z represents NH; and R' represents hydrogen, or optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl.

35. The compound of claim 18, wherein Z represents NH; and R" represents hydrogen, hydroxyl, or optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl; or aminoalkyl, or amino(heteroalkyl); or ZR" taken together represents hydroxyl or a heterocycle comprising Z and a secondary amine.

36. The compound of claim 18, wherein Z represents NH; and Ar represents phenyl.

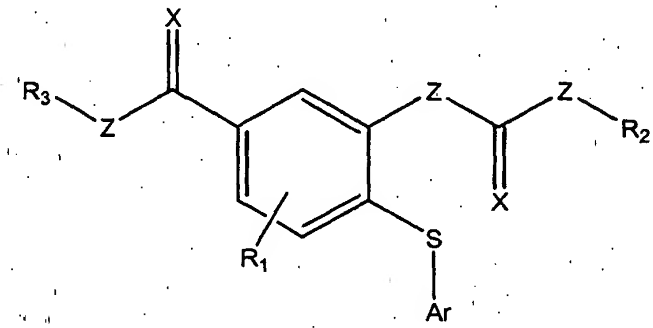
37. The compound of claim 18, wherein Z represents NH; and Ar' represents optionally substituted phenyl, pyridyl, or quinolinyl.

38. The compound of claim 18, wherein X represents O; Z represents NR; R' represents hydrogen, or optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl; R" represents hydrogen, hydroxyl, or optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl; or aminoalkyl, or amino(heteroalkyl); or ZR" taken together represents hydroxyl or a heterocycle comprising Z and a secondary amine; Ar represents phenyl; and Ar' represents optionally substituted phenyl, pyridyl, or quinolinyl.

39. The compound of claim 18, wherein X represents O; Z represents NH; R' represents hydrogen, or optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl; R" represents hydrogen, hydroxyl, or optionally substituted alkyl, alkenyl, alkynyl, aryl, or

heteroaryl; or aminoalkyl, or amino(heteroalkyl); or ZR" taken together represents hydroxyl or a heterocycle comprising Z and a secondary amine; Ar represents phenyl; and Ar' represents optionally substituted phenyl, pyridyl, or quinolinyl.

40. The compound represented by general structure **3**:



3

wherein

Ar is selected from the group consisting of optionally substituted aromatic and heteroaromatic moieties;

X represents independently for each occurrence O, S, Se, NR, PR or AsR;

Z represents independently for each occurrence O, S, Se, NR, PR or AsR;

R represents independently for each occurrence hydrogen, optionally substituted alkyl, aryl, or heteroaryl; or formyl, acyl, sulfonyl, -Si(alkyl)$_d$(aryl)$_g$, -Sn(alkyl)$_d$(aryl)$_g$, or -(CH$_2$)$_m$R$_8$;

R_1 may be absent, or may be present any number of times consistent with the limitations imposed by the rules of valence;

R_1, when present, represents independently for each occurrence halogen, alkyl, alkenyl, alkynyl, hydroxyl, alkoxyl, silyloxy, amino, nitro, sulfhydryl, alkylthio, imine, amide, phosphoryl, phosphonate, phosphine, carbonyl, carboxyl, carboxamide, anhydride, silyl, thioalkyl, alkylsulfonyl, arylsulfonyl, selenoalkyl, ketone, aldehyde, ester, heteroalkyl, nitrile, guanidine, amidine, acetal, ketal, amine oxide, aryl, heteroaryl, azide, aziridine, carbamate, epoxide, hydroxamic acid, imide, oxime, sulfonamide, thioamide, thiocarbamate, urea, thiourea, or -(CH$_2$)$_m$R$_8$;

R_2 represents hydrogen, optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl; or -(CH$_2$)$_m$R$_8$;

R_3 represents hydrogen, hydroxyl, or optionally substituted alkyl, alkenyl, alkynyl, aryl, or heteroaryl; or aminoalkyl, or amino(heteroalkyl); or ZR_3 taken together represents hydroxyl or a heterocycle comprising Z and a secondary amine;

R_8 represents independently for each occurrence an optionally substituted aryl, cycloalkyl, cycloalkenyl, heterocycle or polycycle;

d and g are integers independently selected from the range 0 to 3 inclusive, whose sum is 3; and

m is an integer in the range 0 to 8 inclusive.

41. The compound of claim 40, wherein X represents O.

42. The compound of claim 40, wherein Z represents NR.

43. The compound of claim 40, wherein Z represents NH.

44. The compound of claim 40, wherein R_1 is absent.

45. The compound of claim 40, wherein R_2 represents optionally substituted aryl or heteroaryl.

46. The compound of claim 40, wherein R_2 represents 4-trifluorophenyl.

47. The compound of claim 40, wherein Ar represents optionally substituted phenyl, pyridyl, or quinolinyl.

48. The compound of claim 40, wherein X represents O; and Z represents NR.

49. The compound of claim 40, wherein X represents O; and Z represents NH.

50. The compound of claim 40, wherein X represents O; and R_1 is absent.

51. The compound of claim 40, wherein X represents O; and R_2 represents optionally substituted aryl or heteroaryl.

52. The compound of claim 40, wherein X represents O; and R_2 represents 4-trifluorophenyl.

53. The compound of claim 40, wherein X represents O; and Ar represents optionally substituted phenyl, pyridyl, or quinolinyl.

54. The compound of claim 40, wherein Z represents NR; and R_1 is absent.

55. The compound of claim 40, wherein Z represents NR; and R_2 represents optionally substituted aryl or heteroaryl.

56. The compound of claim 40, wherein Z represents NR; and R_2 represents 4-trifluorophenyl.

57. The compound of claim 40, wherein Z represents NR; and Ar represents optionally substituted phenyl, pyridyl, or quinolinyl.

58. The compound of claim 40, wherein Z represents NH; and R_1 is absent.

59. The compound of claim 40, wherein Z represents NH; and R_2 represents optionally substituted aryl or heteroaryl.

60. The compound of claim 40, wherein Z represents NH; and R_2 represents 4-trifluorophenyl.

61. The compound of claim 40, wherein Z represents NH; and Ar represents optionally substituted phenyl, pyridyl, or quinolinyl.

62. The compound of claim 40, wherein X represents O; Z represents NR; R_1 is absent; R_2 represents optionally substituted aryl or heteroaryl; and Ar represents optionally substituted phenyl, pyridyl, or quinolinyl.

63. The compound of claim 40, wherein X represents O; Z represents NH; R_1 is absent; R_2 represents 4-trifluorophenyl; and Ar represents optionally substituted phenyl, pyridyl, or quinolinyl.

64. The compound of claim 40, wherein X represents O; Z represents NR; R_1 is absent; R_2 represents 4-trifluorophenyl; and Ar represents optionally substituted phenyl, pyridyl, or quinolinyl.

65. The compound of claim 40, wherein X represents O; Z represents NH; R_1 is absent; R_2 represents optionally substituted aryl or heteroaryl; and Ar represents optionally substituted phenyl, pyridyl, or quinolinyl.

66. The compound of claim 1, 18, or 40, wherein said compound is a ligand for a G-protein-coupled receptor.

67. The compound of claim 1, 18, or 40, wherein said compound is a ligand selective for one family of G-protein-coupled receptors.

68. The compound of claim 67, wherein the G-protein-coupled receptor is an adenosine receptor.

69. The compound of claim 67, wherein the G-protein-coupled receptor is a 5-HT receptor.

70. The compound of claim 68, wherein said compound is a ligand selective for one subtype of adenosine receptor.

71. The compound of claim 69, wherein said compound is a ligand selective for one subtype of 5-HT receptor.

72. A pharmaceutical formulation comprising a compound of claim 1, 18, or 40.

73. A pharmaceutical formulation comprising a compound of claim 66.

74. A pharmaceutical formulation comprising a compound of claim 67.

75. A pharmaceutical formulation comprising a compound of claim 68.

76. A pharmaceutical formulation comprising a compound of claim 69.

77. A pharmaceutical formulation comprising a compound of claim 70.

78. A pharmaceutical formulation comprising a compound of claim 71.

79. A method of treating an acute or chronic ailment, disease or malady that is at least in part due to a biochemical or physiological process associated with a G-protein-coupled receptor, comprising the step of administering to a patient in need thereof a pharmaceutical formulation of claim 73.

80. A method of treating an acute or chronic ailment, disease or malady that is at least in part due to a biochemical or physiological process associated with one family of G-protein-coupled receptors, comprising the step of administering to a patient in need thereof a pharmaceutical formulation of claim 74.

81. A method of treating an acute or chronic ailment, disease or malady that is at least in part due to a biochemical or physiological process associated with an adenosine receptor, comprising the step of administering to a patient in need thereof a pharmaceutical formulation of claim 75.

82. A method of treating an acute or chronic ailment, disease or malady that is at least in part due to a biochemical or physiological process associated with a 5-HT receptor, comprising the step of administering to a patient in need thereof a pharmaceutical formulation of claim 76.

83. A method of treating an acute or chronic ailment, disease or malady that is at least in part due to a biochemical or physiological process associated with a single subtype of adenosine receptor, comprising the step of administering to a patient in need thereof a pharmaceutical formulation of claim 77.

84. A method of treating an acute or chronic ailment, disease or malady that is at least in part due to a biochemical or physiological process associated with a single subtype of 5-HT receptor, comprising the step of administering to a patient in need thereof a pharmaceutical formulation of claim 78.

Figure 1

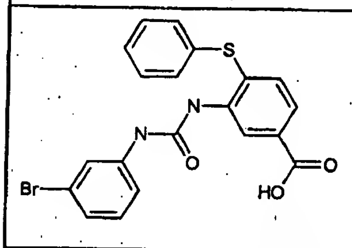
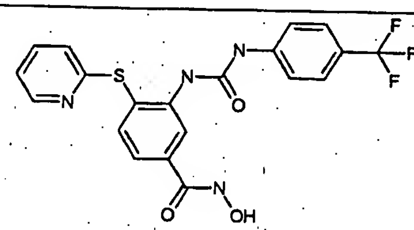
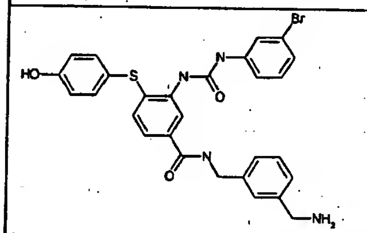
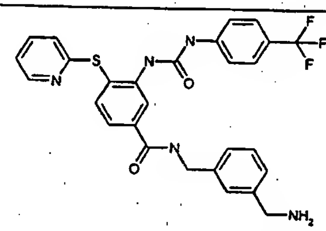
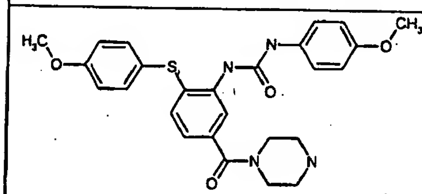
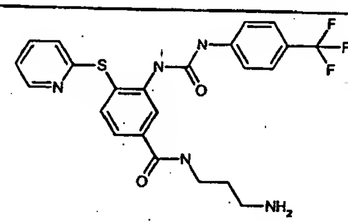
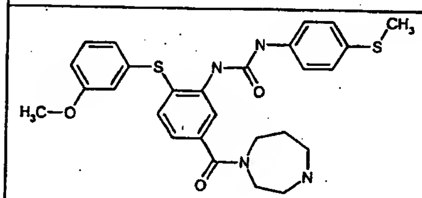
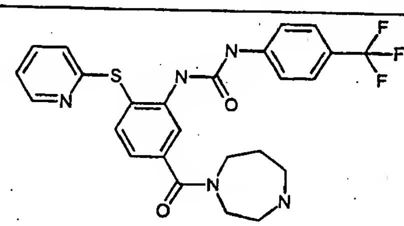
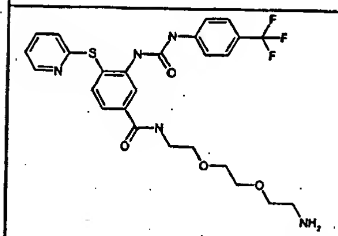
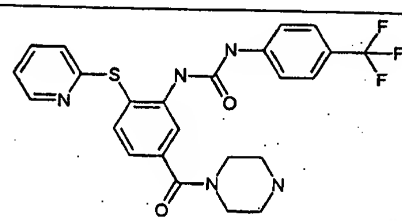
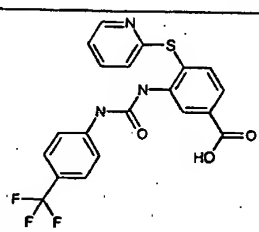
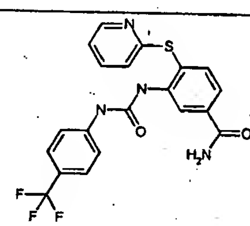
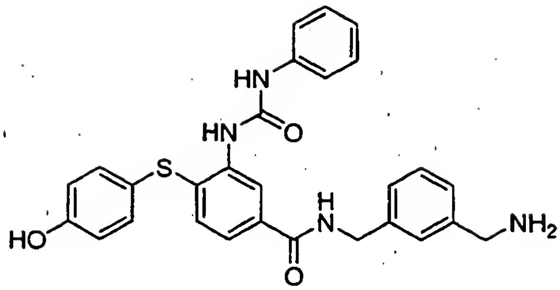
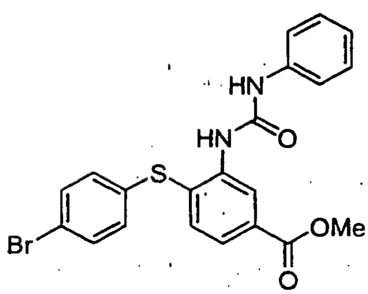
COMPOUND	MIC vs. MRSA (μg/mL)	COMPOUND	MIC vs. MRSA (μg/mL)
	12.5		>25
	6.5		>25
	>25		>25
	25		>25
	12.5		>25
	12.5		25

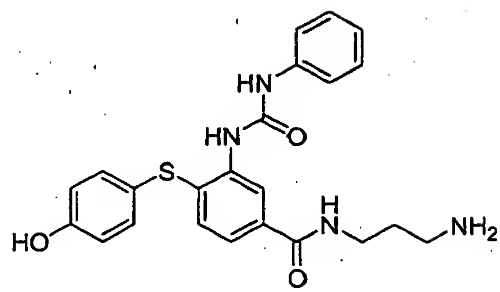
Figure 2



Receptor	K_i
A_1 (rat)	>10 µM
A_{2a} (human)	>10 µM
A_3 (human)	0.480 µM



Receptor	K_i
A_1 (rat)	>10 µM
A_{2a} (human)	>10 µM
A_3 (human)	2.7 µM



Receptor	K_i
A_1 (rat)	>10 µM
A_{2a} (human)	>10 µM
A_3 (human)	0.911 µM

Figure 3

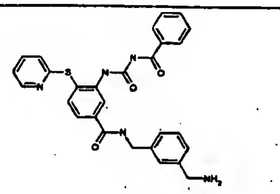
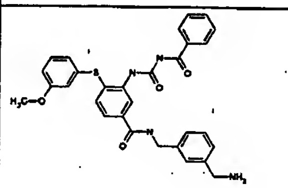
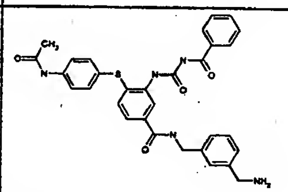
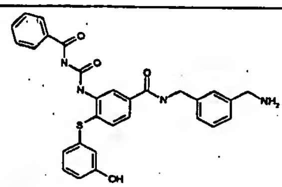
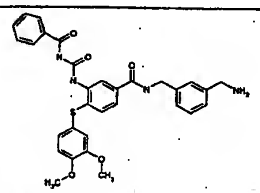
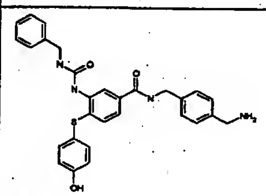
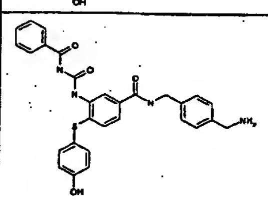
Compound #	STRUCTURE	A1 (Human) IC_{50} (nM)	A2a (Human) IC_{50} (nM)	A2b (Human) IC_{50} (nM)	A3 (Human) IC_{50} (nM)
30		>1000	>1000	>1000	>1000
31		>1000	>1000	<500	<1000
32		>1000	>1000	>1000	>1000
33		>1000	>1000	>1000	>1000
34		>1000	>1000	>1000	<1000
35		>1000	>1000	>1000	>1000
36		>1000	>1000	>1000	<1000

Figure 4

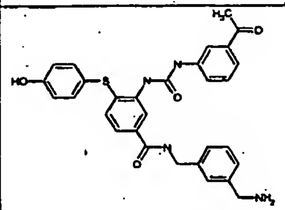
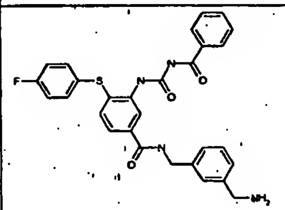
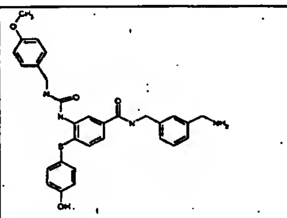
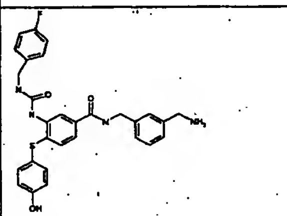
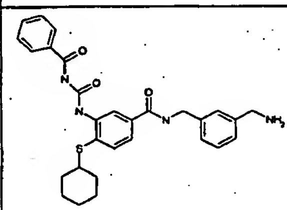
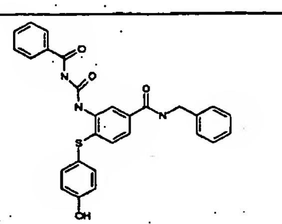
Comp und #	STRUCTURE	A1 (Human) IC_{50} (nM)	A2a (Human) IC_{50} (nM)	A2b (Human) IC_{50} (nM)	A3 (Human) IC_{50} (nM)
37		>1000	>1000	>1000	>1000
38		>1000	>1000	>1000	>1000
39		>1000	>1000	>1000	>1000
40		>1000	>1000	>1000	>1000
41		>1000	>1000	>1000	>1000
42		>1000	>1000	>1000	<1000

Figure 5

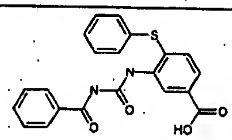
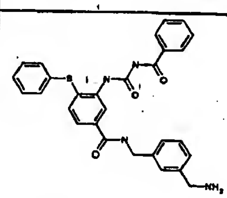
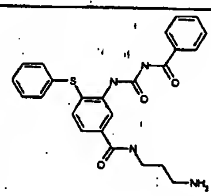
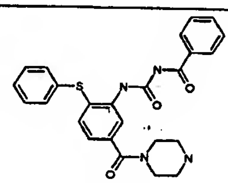
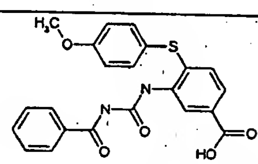
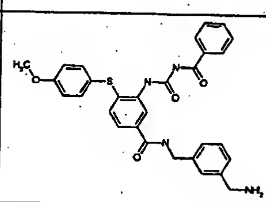
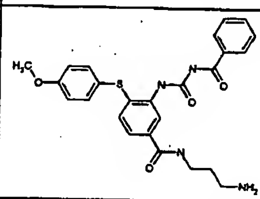
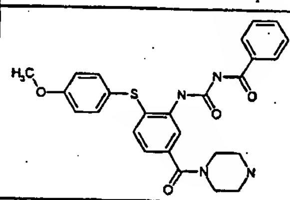
Compound #	Structure	A1 (Human) IC_{50} (nM)	A1 (Rat) IC_{50} (nM)
50		>10000	>10000
51		<5000	
52		<10000	
53		<1000	<5000
54		>10000	>10000
55		<10000	
56		<5000	<10000
57		<10000	

Figure 6

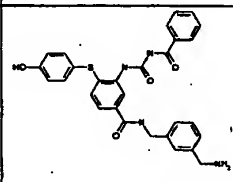
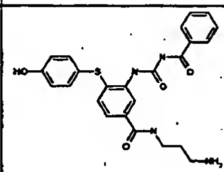
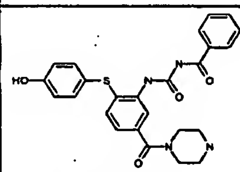
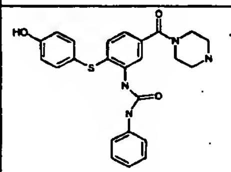
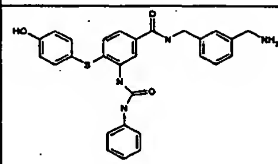
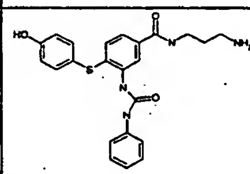
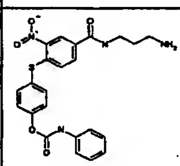
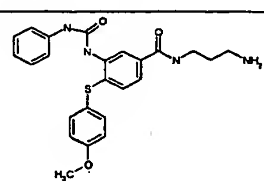
Compound #	Structure	A1 (Human) IC_{50} (nM)	A1 (Rat) IC_{50} (nM)
58		<1000	1160
59		<5000	<5000
60		<1000	<1000
61		>10000	>10000
62		>10000	>10000
63		>10000	>10000
64		>10000	>10000
65		>10000	>10000

Figure 7

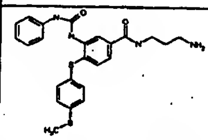
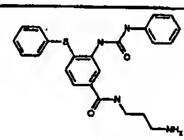
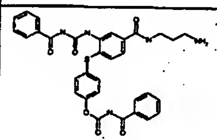
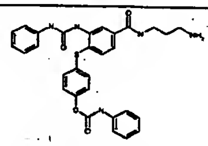
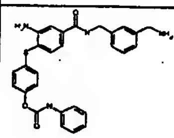
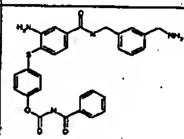
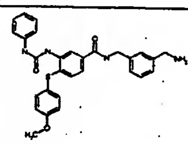
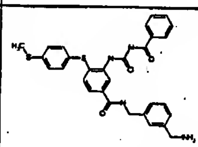
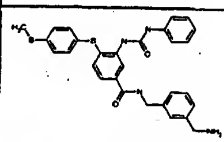
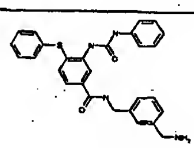
Compound #	Structure	A1 (Human) IC_{50} (nM)	A1 (Rat) IC_{50} (nM)
66		>10000	>10000
67		>10000	>10000
68		<5000	<10000
69		>10000	>10000
70		>10000	>10000
71		>10000	>10000
72		>10000	>10000
73		>10000	>10000
74		>10000	>10000
75		>10000	>10000

Figure 8

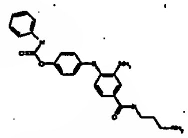
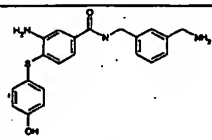
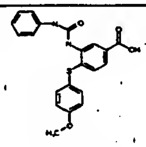
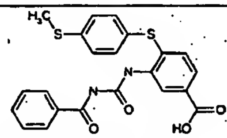
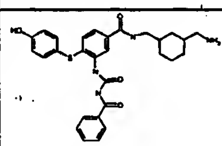
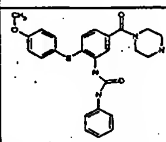
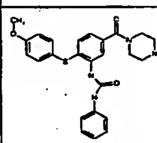
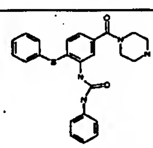
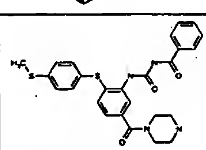
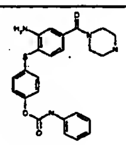
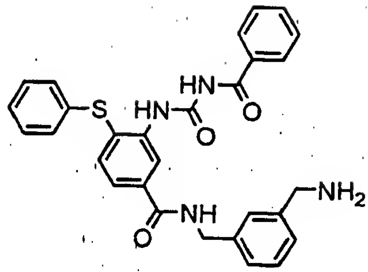
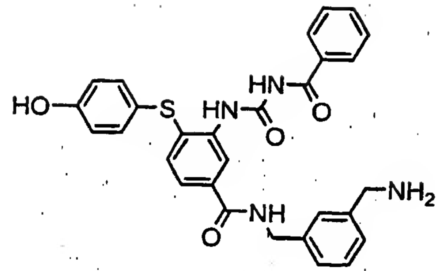
Compound #	Structur	A1 (Human) IC_{50} (nM)	A1 (Rat) IC_{50} (nM)
76		>10000	>10000
77		>10000	>10000
78		>10000	>10000
79		>10000	>10000
80		<500	<500
81		>10000	>10000
82		>10000	>10000
83		>10000	>10000
84		>10000	>10000
85		>10000	>10000

Figure 9



A



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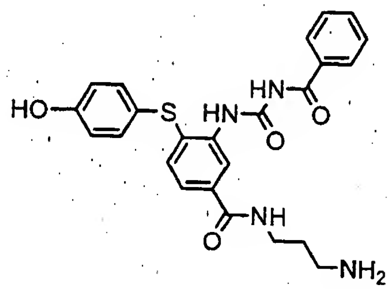
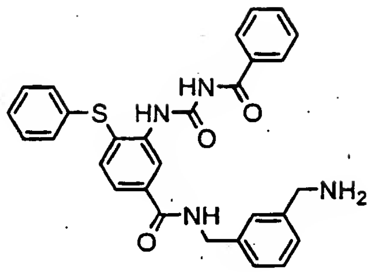


C

Compound	A	B	C
Solubility (μM, water)	2	3	22
Log D (pH 7.4)	4.08	3.5	1.93
Permeability TC 7 Cells (Papp 10^{-6} cm/s)	15.9	0.34	0.14
Metabolic Stability (% remaining)	102	94	101
CYP1A2 (% inhib.)	48	35	22
CYP2C9 (% inhib.)	98	98	78
CYPC19 (% inhib.)	55	58	33

Figure 10



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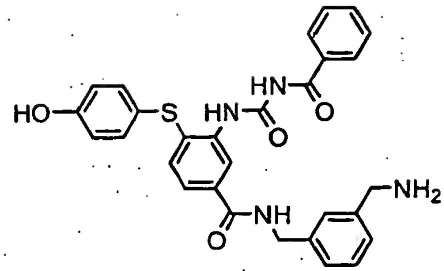

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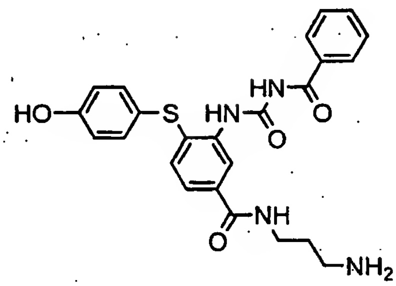
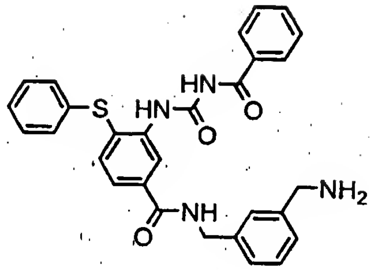


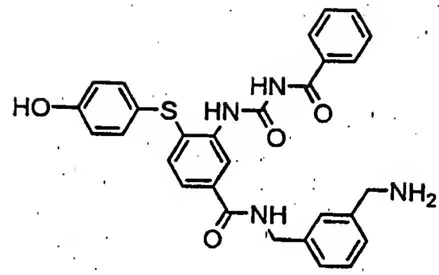
C

Compound	A	B	C
CYP2D6 (% inhib.)	74	104	101
CYP3A4 (% inhib.)	88	89	49
Cell Viability (% inhib.)	94	<10	<10
Membrane Integrity (% inhib.)	83	<10	<10

Figure 11



A



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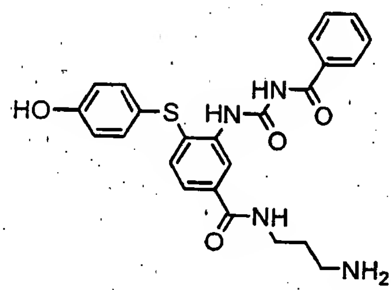

C

Compound	A	B	C
Capsase-3 Activity (% induction)	<10	<10	<10
Protein Synthesis (% inhib.)	64	<10	<10
DNA Synthesis (% inhib.)	99	26	22
Glutathione Level (% control)	-125	40	<10

INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/US 00/07903

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07C323/62 C07D213/70 C07D243/06 C07D295/192 A61K31/10
A61K31/17 A61K31/192 A61K31/435 A61K31/495 A61K31/551

According to International Petent Classification (IPC) or to both national classification end IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07C C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (neme of data base and, where practical, search terms used)

EPO-Internal, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indicetion, where eppropriete, of the relevant passages	Relevent to claim No.
X	DE 36 02 016 A (BAYER AG) 11 June 1987 (1987-06-11) examples 284,285 ---	1-65
A	WO 97 17325 A (FARMAK A S ;POLIVKA ZDENEK (CZ); DOBROVSK KAREL (CZ); SILHANKOVA A) 15 May 1997 (1997-05-15) the whole document ---	1-84
A	EP 0 707 007 A (MERCK PATENT GMBH) 17 April 1996 (1996-04-17) the whole document -----	1-84

☐ Further documents ere listed in the continuation of box C.

☒ Patent family members ere listed in annex.

° Speciel categories of cited documents :

"A" document defining the general state of the ert which is not considered to be of particular relevence
"E" earlier document but published on or efter the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (es specified)
"O" document referring to en oral disclosure, use, exhibition or other means
"P" document published prior to the international filing dete but later than the priority date cleimed

"T" later document published after the Intemetional filing date or priority date end not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cennot be considered to involve an inventive step when the document is taken elone
"Y" document of particular relevence; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to e person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the intemational search	Date of mailing of the intemational search report
25 July 2000	07.08.00

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Petentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Goetz, G

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 00 /07903

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1-84 (see further information)

Present claims 1 to 84 relate to an extremely large number of possible compounds. In particular the claimed general formulae of present independent claim 1 which consists of only variables and claim 18 which consists of only one non-variable atom (sulfur atom) and claim 40 which consists only of a very small non-variable part, hardly represent a permissible generalisation of the compounds given in figures 1 to 11. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is therefore to be found, however, for only a very small proportion of the compounds claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the compounds given in figures 1 to 11.
No special search effort can be made for searching unduly wide or speculative claims; the vast number of compounds falling within the limits of the present claims precludes a comprehesive search.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 00/07903

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 79 to 84 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: 1-84 (see further information)
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 00/07903

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
DE 3602016 A	11-06-1987	AU 592839 B	25-01-1990
		AU 6587786 A	11-06-1987
		BR 8605956 A	15-09-1987
		DK 583986 A	06-06-1987
		EP 0226837 A	01-07-1987
		HU 45372 A	28-07-1988
		US 4871387 A	03-10-1989
		DD 265317 A	01-03-1989
		JP 62132862 A	16-06-1987
		ZA 8609159 A	26-08-1987
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		EP 0859757 A	26-08-1998
		HU 9901136 A	30-08-1999
		SK 46798 A	09-09-1998
EP 0707007 A	17-04-1996	AU 703637 B	25-03-1999
		AU 3421895 A	26-04-1996
		BR 9504379 A	27-05-1997
		CA 2160447 A	15-04-1996
		CN 1130180 A	04-09-1996
		CZ 9502661 A	17-04-1996
		FI 954874 A	15-04-1996
		HU 75644 A	28-05-1997
		JP 8225501 A	03-09-1996
		NO 954080 A	15-04-1996
		PL 310932 A	15-04-1996
		SK 126695 A	05-06-1996
		TR 960308 A	21-06-1996
		US 5767132 A	16-06-1998
		ZA 9508673 A	22-05-1996